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Metabolism of Radioactive DDT by the Madeira Roach and European Corn Borer[1,2]

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The only identified metabolite of DDT produced by insects is DDE, 2,2-*bis*-(*p*-chlorophenyl)-1,1-dichloroethylene (Hoskins & Gordon, 1956) but unidentified metabolites have been reported by many authors (Ferguson & Kearns 1949, Sternburg *et al.* 1950, Sternburg & Kearns 1950, 1952a, 1952b, Babers & Pratt 1953, Babers & Roan 1953, Butts *et al.* 1953, Tahori & Hoskins 1953, Robbins & Dahm 1955, Terriere & Schonbrod 1955).

The experiments reported here were designed to study the absorption, metabolism, and excretion of radioactive DDT by the adult, female Madeira roach, *Leucophaea maderae* (F.) and by the fifth instar European corn borer larva, *Pyrausta nubilalis* (Hbn.).

MATERIALS AND METHODS.—*Radioactive Insecticides.*—The *p,p'*-C^{14}-ring-labeled DDT[3] used in these studies had a specific activity of 0.75 millicurie per millimole and an average observed activity of 2195 counts per minute (cpm) per microgram (μg.) when assayed on a windowless, gas-flow GM counter.[4] The *p,p'*-C^{14}-ring-labeled DDE[5] used had an observed activity of about 145 cpm/μg. This compound was used only as a standard for paper chromatographic analysis. Both radioactive materials were found to be pure when checked by paper chromatography. Purity of the radioactive DDT was confirmed also by the colorimetric method of Schechter *et al.* (1945).

Handling of Insects.—Laboratory-reared, adult, female Madeira roaches were used in these experiments. The cockroaches were reared by the method described by Dahm (1955) and sexed according to the criteria set forth by Gurney (1953).

The cockroaches were anesthetized lightly with carbon dioxide before treatment. Radioactive DDT was applied as an acetone solution with a 10-microliter (*ul.*) pipet to the ventral thoracic region of the cockroaches. Evaporation of the acetone was hastened by blowing on the treated area. Twenty treated cockroaches were placed in a wide-mouth, one-gallon jar. Food and water were added to each jar and the top covered with $\frac{1}{4}$-inch hardware cloth, and the jars were inverted over glass dishes into which the feces fell. Cockroaches treated with 10 μl. of acetone and handled in a like manner were used as controls. All cockroaches were held at a temperature of $80 \pm 2°$ F.

Feces from the cockroaches were collected daily, weighed, and stored in closed bottles until used. Dead cockroaches were removed each day (less than 24 hours after dying).

The experimental data were obtained from two groups of cockroaches treated with radioactive DDT. Group I consisted of 20 cockroaches treated with a calculated 100 μg. (222,500 cpm) of DDT per cockroach and group II consisted of 100 cockroaches treated with a calculated 66 μg. (144,500 cpm) of DDT per cockroach.

Although the first instar European corn borer larvae is the stage most often controlled by DDT in the field (Turner 1945), most of the experiments were done with fifth instar larvae which were easier to handle. During the summer of 1955, second or later instar larvae were collected in the field from insecticide-free corn. The larvae were reared to the desired size in petri dishes in the laboratory. Damp filter papers were placed in the bottom of the dishes to insure high humidity, and sections of fresh green beans were used for food (Mathes 1936). From 10 to 15 larvae were placed in each dish and fresh food added as needed.

Preliminary experiments were conducted with non-radioactive DDT to determine dosages the larvae could tolerate. Larvae were exposed to deposits of DDT in shell vials (Hoskins *et al.* 1952) and were treated topically with acetone solutions of DDT.

Only fifth instar larvae were used in the radioactive DDT metabolism studies. A dosage of 10.1 μg. of radioactive DDT, in an acetone solution, was applied topically to the dorsal surface of each larva with a 1-μl. pipet. To facilitate handling, the larvae were anesthetized lightly with carbon dioxide just before treatment. The average weight of some groups of fifth instar larvae was determined. After treatment each larva was placed in a 1.4×5 cm., screen-capped vial. A piece of green bean was placed in each vial for food, and damp paper toweling was laid over the top of the upright vials to maintain a high

PAID PAPER.

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[2] Part of a thesis submitted by Donald A. Lindquist to Iowa State College in partial fulfillment of the requirements for the degree of Master of Science. Thanks are due Dr. T. A. Brindley and Mr. F. F. Dicke for making it possible to collect the European corn borer larvae at the Ankeny, Iowa, European Corn Borer Laboratory and suggesting handling techniques.

[3] Obtained from the Oak Ridge National Laboratory (Carbide and Carbon Chemicals Co.), Oak Ridge, Tenn.

[4] Model D-46A, Nuclear Instrument and Chemical Corp., Chicago, Ill.

[5] Obtained from the Soil and Water Conservation Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md.

humidity. All experiments were run at temperatures of 85 to 95° F.

Extraction Procedures.—Each day's dead cockroaches were rinsed four or five times with acetone immediately after removing them from the cages. Extraction of the radioactivity in the cockroach feces was accomplished by soaking the feces for about 72 hours in about 15 ml. of a mixture of acetone, water, and sulfuric acid (AWA).[6]

Groups of European corn borer larvae were rinsed four times with either petroleum ether (b.p. 60–70° C.) or acetone at definite intervals after treatment to remove the external DDT. The larvae then were ground in a mortar with sand and anhydrous sodium sulfate. Extraction of internal DDT and metabolites was accomplished by mechanically shaking the ground mixture with carbon tetrachloride for an hour. The radioactivity in the feces was extracted by the same method. Finally, the holding cages and beans were rinsed twice with carbon tetrachloride to remove any DDT that might have fallen or been rubbed off the larvae.

Analytical Methods.—Radioassays were made with a windowless, gas-flow GM counter[4] and a conventional scaler.[7] A piece of C^{14}-labeled polystyrene (approximately 4,500 cpm), glued to a planchet, was used as a reference standard. Radioassay samples were prepared in triplicate on aluminum planchets and were counted long enough to attain a standard error of $\pm 5\%$ (Calvin *et al.* 1949).

The colorimetric method of Schechter *et al.* (1945) for determining DDT and DDE was used in these experiments. The method of Knudsen *et al.* (1940) for resolving a two-component color system was used to measure the amounts of DDT and DDE in a mixture of the two compounds. This method previously had been used successfully by Wichmann *et al.* (1946) and Mattson *et al.* (1953).

The internal extracts of the treated corn borer larvae were passed through a sulfuric acid-Celite column (Davidow 1950) to remove the fat before employing the colorimetric analysis. Known amounts of DDT and DDE were always included in the analyses.

For the paper chromatographic analyses (Winteringham *et al.* 1950, as modified by Jackson & Hopkins 1952, and Robbins & Dahm 1955) a cylindrical glass chamber 12 inches in diameter and 48 inches high was used. The mobile solvent consisted of 75% ethanol (95%), 20% water, and 5% ammonium hydroxide (V/V). The immobile solvent, Vaseline,[8] was applied to 1-inch strips of Whatman No. 1 filter paper[9] by dipping the strips in a 2.5% solution of Vaseline in diethyl ether (W/W).

The location (R_f value)[10] of the radioactivity on the chromatograms was determined by passing the strips beneath a Micromil, thin-window GM tube[11] connected with a rate meter[12] and an automatic recorder.[13] The Micromil counter was about 80% as efficient as the windowless, gas-flow counter mentioned earlier.[4] The window end of the GM tube rested on an aluminum base provided with a channel to receive the paper chromatogram and an adjustable slit to vary the area of the chromatogram scanned. The chromatogram passed about 1.5 mm. below the Micromil window. Movement of the chromatogram was effected by taping it to the chart of the recorder. Satisfactory results were obtained with a slit width of 1 cm., a velocity of 6 inches per hour, and rate meter settings of 2% statistical error and a range of 500 cpm. The distribution of radioactivity was determined by measuring the area under each peak on the recording of the chromatogram and under the entire recording (less background) with a planimeter. An average value for three chromatograms was obtained. The accuracy of this method was checked by eluting the radioactivity from chromatograms and radioassaying the eluates. Bull *et al.* (1949) and Redfield & Guzman Barron (1952) used a planimeter in a similar way.

RESULTS.—*Cockroach Experiments.*—The combined acetone rinses of each day's dead cockroaches were radioassayed to determine the rate of absorption of DDT. Babers & Roan (1953) found only DDT in the external rinses of topically treated German cockroaches, therefore chemical analyses of the external rinses were not made. Rates of absorption and excretion of DDT and metabolites by the two groups of radioactive DDT-treated Madeira roches were nearly identical. Therefore, only data from group II (100 cockroaches treated with 66 μg. of DDT per cockroach) will be presented unless otherwise indicated.

About 33% of the DDT was absorbed during the first 24 hours after treatment (fig. 1) and a slower rate of absorption occurred thereafter. The over-all rate of absorption was much slower than reported for the American cockroach (Robbins & Dahm 1955). This undoubtedly was one reason the Madeira roach could tolerate large dosages of DDT. The average weight of adult, female Madeira roaches was about 2.4 gm. Thus, the DDT was applied to cockroaches in groups I and II at approximately 42 and 28 μg./g. of body weight, respectively. The mortality in the two groups, 36 days after treatment, was 25 and 30%, respectively; the mortality among control cockroaches over the same period was 5 and 30%, respectively.

The efficiency of extracting radioactivity from feces by soaking in about 15 ml. of AWA[6] is shown in table 1. Other methods of extractions (soxhlet and shaking) and other solvents such as acetone, carbon tetrachloride,

[6] AWA = 80 ml. acetone, 20 ml. water, and 1 ml. 1 N sulfuric acid.
[7] Model 2105 Decimal Scaler, Berkeley Scientific Division of Beckman Instruments, Inc., Richmond, Calif.
[8] Chesebrough Mfg. Co. Cons'd., New York, N. Y.
[9] W. and R. Balston, Ltd., England.
[10] R_f value = distance traveled by the material divided by distance traveled by the solvent front.
[11] Model D-47, Nuclear Instrument and Chemical Corp., Chicago, Ill.
[12] Model 1615-B, Nuclear Instrument and Chemical Corp., Chicago, Ill.
[13] Model AW, The Esterline-Angus Co., Inc., Indianapolis, Ind.

Table 1.—Extraction of radioactive DDT and metabolites from 138 mg. of Madeira roach feces by soaking in AWA[a] and subsequent grinding with AWA.

PROCEDURE	RADIOACTIVITY EXTRACTED	
	cpm (corr.)	Per Cent
1. Acetone rinse	2,300	7
2. Soaking in 15 ml. AWA		
a. 18 hrs.	28,900	81
b. 48 hrs. additional	3,100	9
3. Grinding with AWA	800	2
4. Residue	300	1

[a] AWA = 80 ml. acetone, 20 ml. water, and 1 ml. 1 N sulfuric acid.

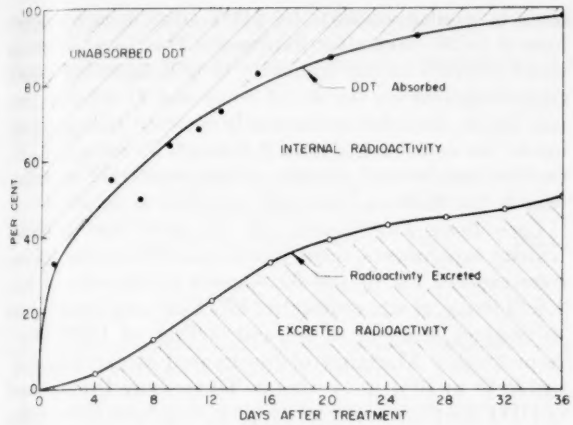

FIG. 1.—Absorption of radioactive DDT and excretion of radioactivity in feces following topical application of DDT to 100 Madeira roaches at the rate of 66 μg. per roach.

diethyl ether, acetonitrile, and water were less effective than AWA in extracting the radioactivity from the feces.

The accumulative per cent of the total dose excreted is shown in figure 1. The maximum daily rate of excretion (7,100 cpm, equivalent to 3.2 μg. of DDT/cockroach/day) occurred 11 days after treatment. A total of 50% of the radioactivity applied was excreted over the 36-day experiment. This was less than the radioactivity excreted by the American cockroach under similar conditions (Robbins & Dahm 1955).

The total internal radioactivity of the DDT-treated cockroaches in group II is shown in figure 2. These data were obtained by subtracting the per cent excreted from the per cent absorbed (fig. 1). The latter curve was based on the amount of DDT remaining on the cuticle of the 30 cockroaches that succumbed during the 36-day excretion experiment. It appears from figures 1 and 2 that after about 6 days a balance is maintained between radioactive DDT absorbed through the cuticle and radioactivity excreted in the feces. From 6 to 36 or more days after treatment 50% of the DDT applied topically was retained within the cockroaches as DDT and metabolites. The same condition was true of cockroaches in group I, except that about 40% of the DDT applied remained inside the cockroaches from 10 to 50 days after treatment.

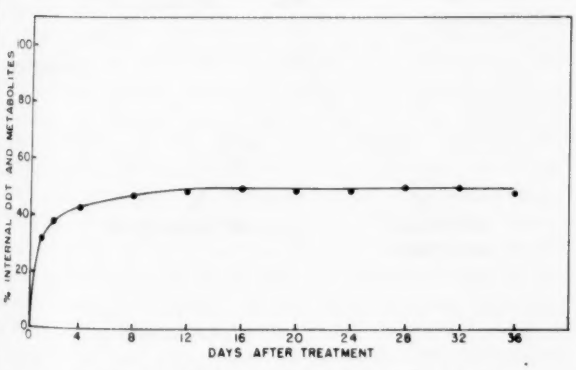

FIG. 2.—Internal DDT and metabolites in Madeira roaches treated topically with 66 μg. of radioactive DDT per insect.

These figures indicate that radioactivity equivalent to 30 to 40 μg. of DDT was inside the cockroaches for extended periods of time.

Colorimetric analyses of fecal extracts were of little value because of interfering materials extracted from the feces along with the radioactive compounds. Furthermore, less than 50% of the radioactivity in the extracts was carried through the analyses.

Reversed-phase paper chromatography was used extensively to separate the radioactive compounds extracted from the feces. Radioactive DDT had an R_f value of 0.564 ± 0.015 (23 runs) and radioactive DDE had an R_f value of 0.494 ± 0.015 (16 runs). From three to eight chromatograms were used to determine the R_f values of the radioactive compounds in the fecal extracts. The mean distance the solvent front moved for all chromatograms was about 76 cm.

The per cent radioactivity in each peak on the chromatograms was determined by use of a planimeter. A check of this method by elution and radioassay gave results of 40 and 64% for the elution method compared with 36 and 68%, respectively, for the planimeter method.

Radioactive AWA extracts of feces from the 100 cockroaches in group II collected during three periods, 0 to 24 hours, 48 to 72 hours, and 21 to 23 days after treatment of the cockroaches, were analyzed by paper chromatography to determine if the nature and quantity of the compounds excreted during these three periods were the same. Both DDT and DDE were excreted during all three periods (fig. 3). All percentages of radioactivity were determined by the planimeter method. DDT was the major radioactive compound excreted during the 24 hours immediately after treatment, accounting for about 53% of the radioactivity. DDE accounted for about 8% and a long smeared spot (B+C) for about 23%. The radioactivity excreted during the later two periods consisted of DDT, DDE, and metabolites A, B, and C. During these periods, metabolite C accounted for more than one-half of the radioactivity excreted. This metabolite had an R_f value of 0.871 to 0.889 (table 2). This experiment indicated that: (a) The per cent of DDT, DDE,

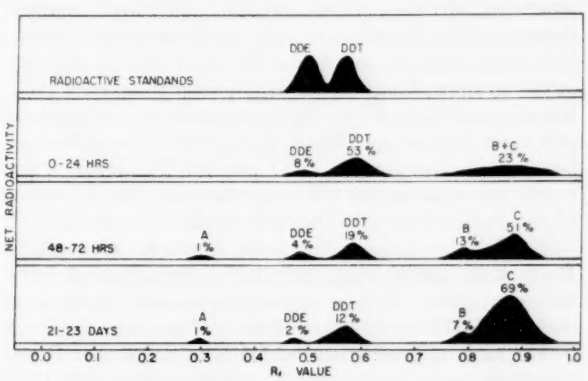

FIG. 3.—Radioassay of paper chromatograms of radioactive DDT, DDE, and extracts of Madeira roach feces excreted from 0 to 24 hours, 48 to 72 hours, and 21 to 23 days after treatment with 66 μg. of radioactive DDT per insect. Percentages of radioactivity under the curves as determined with a planimeter are included.

Table 2.—R_f values and standard deviations from paper chromatograms of AWA extracts of Madeira roach feces excreted from 0 to 24 hrs., 48 to 72 hrs., and 21 to 23 days after topical treatment with 66 μg. of radioactive DDT per insect.[a]

Period of Fecal Collection	R_f Values				
	Metabolite A	DDE[b]	DDT[b]	Metabolite B	Metabolite C
0 to 24 hrs.		0.480 ±0.013	0.584 ±0.021		0.871 ±0.026
48 to 72 hrs.	0.298 ±0.010	0.481 ±0.019	0.582 ±0.021	0.798 ±0.028	0.889 ±0.026
21 to 23 days	0.297 ±0.021	0.476 ±0.011	0.570 ±0.012	0.791 ±0.021	0.873 ±0.022

[a] See also figure 3.
[b] Average R_f values for DDE and DDT standards were 0.494 and 0.564 respectively.

and metabolite B excreted decreased with time; (b) The per cent of metabolite C increased with time and probably reached a constant value about 6 days after treatment; (c) Metabolite A was present in a very small amount in the two later periods and may have been excreted during the first 24 hours, but was not detected because of the small amount of radioactivity present; (d) Metabolite A appeared to be less polar and metabolites B and C appeared to be more polar than DDT and DDE.

It is unlikely that any one of the unidentified metabolites is DDA. The R_f value of DDA as reported by Jackson & Hopkins (1952) was 0.80 which would exclude metabolite A. Robbins & Dahm (1955) reported DDT metabolites produced by the American cockroach similar to B and C, but concluded that neither was DDA. Failure to extract metabolites B and C by alkaline solutions also indicated that neither was DDA.

An attempt was made to separate the internal DDT metabolites by paper chromatography to determine their order of appearance. These experiments yielded no useful information because the large amount of fat in the extracts caused the radioactivity to smear over the entire chromatogram.

Definite relationships have been proven experimentally between the R_f values of certain organic compounds separated by paper chromatography and their chemical structure. The R_f values of fatty acids and their derivatives show a regular increase in R_f value with an increase in the number of carbon atoms (Lederer & Lederer 1953, Block *et al.* 1955). The same is generally true of alcohols and their derivatives (Block *et al.* 1955). The difference between the R_f values of DDT and DDE in figure 4 was nearly the same as the difference between the R_f values of metabolites B and C. Thus it is possible that both DDT and DDE are conjugated with identical compounds. Butts *et al.* (1953) suggested that a derivative of DDT was conjugated with a carbohydrate and excreted as such by DDT-treated American cockroaches.

The ratio of percentages of DDE to metabolite B was nearly the same in the extracts of feces collected 48 to 72 hours and 21 to 23 days after treatment (4:13 and 2:7 in fig. 3), and the ratio of percentages of DDE to DDT was roughly the same in the extracts of feces collected during all three periods (8:53, 4:19, and 2:12 in fig. 3). The same radioactive compounds probably are excreted in feces in similar amounts by DDT-treated cockroaches from 6 to 36 days after treatment. This assumption is based not only on the similarity of radioassays of paper chromatograms for the 48–72 hours and 21–23 day periods (fig. 3), but also on the nearly constant radioactivity inside the cockroaches from 6 through 36 days (fig. 2). On this basis several possible pathways of DDT metabolism in the Madeira roach are proposed in figure 4.

Corn Borer Experiments.—In the shell vial residual toxicity experiments, third, fourth, and fifth instar larvae were exposed for 10 and 30 minutes to deposits of 0.1, 1, and 10 mg. of non-radioactive DDT per vial (equivalent to residues of about 3, 30, and 300 μg. of DDT/cm.^{2}, respectively). Mortality of the treated larvae was negligible (0 to 10% in 72 hours). To measure the amount of DDT the third instar larvae picked up from these vials, 10 larvae were exposed for 10 minutes in a vial coated with a residue of 10 mg. of DDT. The larvae were then rinsed four times in acetone, the acetone evaporated, and the residue analyzed for DDT. An average of 58 μg. of DDT per larva was recovered. This seemed to be a tremendous amount of DDT to result in so little mortality. Ten more third instar larvae were treated in the same manner and held 22 hours. The larvae then were rinsed with acetone and extracted as previously described. Colorimetric analyses of the external rinse and internal extract showed 1.2 μg. and 0.1 μg. of DDT per larva, respectively. A trace of DDE was found in the internal extract. It was apparent that the larvae were either absorbing the DDT and excreting it and/or metabolites at an extremely fast rate or were rubbing the DDT off of their bodies by crawling around on the filter paper and food in the holding cages. Further experiments demonstrated the second alternative was true.

Topical application of acetone solutions of DDT indicated that a dosage of about 10 μg. of DDT could be

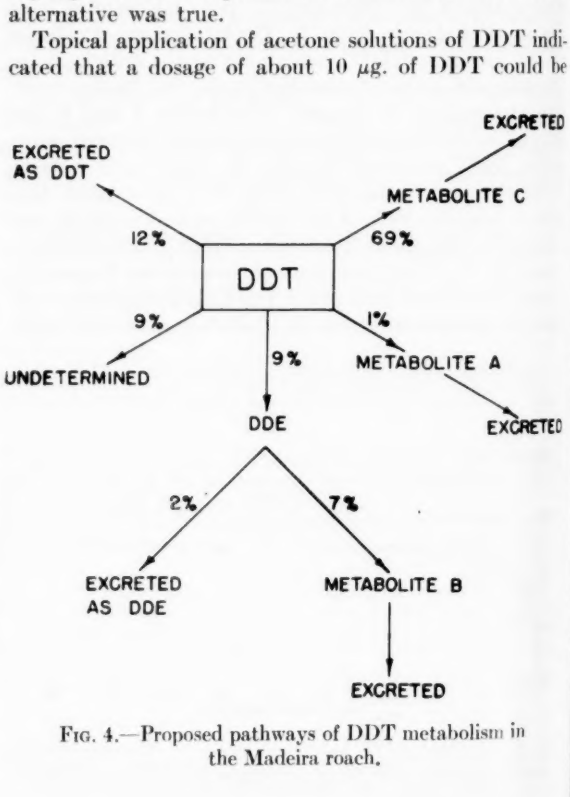

FIG. 4.—Proposed pathways of DDT metabolism in the Madeira roach.

Table 3.—Radioassay and colorimetric analyses of extracts of fifth instar European corn borer larvae topically treated with 10.1 μg. of radioactive DDT per larva.[a]

Hours After Treatment	External DDT[b,c]	DDT Absorbed[b]	Analyses of Internal CCl_4 Extracts (Per Larva)				Absorbed DDT Converted to DDE	Total DDT Accounted for	Range of Mortality[e]
			Radiometric	Colorimetric					
				DDE[d]	DDT	Total			
	(μg./larva)	(Per Cent)	(μg.)	(μg.)	(μg.)	(μg.)	(Per Cent)	(Per Cent)	(Per Cent)
24	8.8	7.9	0.8	0.1	0.7	0.8	13	95	10–20
48	7.1	9.9	1.0	0.3	0.8	1.1	27	80	10–30
72	8.3	10.9	1.1	0.3	0.7	1.0	30	93	20–53
120	6.6	14.8	1.5	0.6	0.8	1.4	43	80	46–69

[a] Values are averages of two groups of 11 to 25 larvae per group.
[b] Radioassay.
[c] Includes external rinse of larvae, rinse of holding cage and beans, and extract of feces.
[d] Converted to molecular equivalents of DDT.
[e] Mortality range of larvae treated with 1 μl. of acetone was 0 to 20%.

tolerated reasonably well by the fifth instar larvae. The average weight of fifth instar larvae was 55 mg. Therefore, the larvae were treated with about 180 μg. of DDT/gm. of body weight. Studies of the metabolism of radioactive DDT by fifth instar European corn borer larvae are summarized in table 3.

The rinses from (a) the larvae and (b) the holding cages and beans, and extracts from (c) the rinsed larvae and (d) feces were analyzed radiometrically. All extracts and rinses, except the external larval rinse, were analyzed for DDT and DDE colorimetrically.

Analyses of the holding cage and bean rinses showed that only DDT was present. These rinses accounted for 39 to 52% of the DDT originally applied to the larvae. The fecal extracts contained considerable but variable amounts of DDT (4 to 21% of the applied dose) but only occasional traces of DDE. The latter might have been due to analytical error. Since both DDT and DDE were present inside the larvae in significant amounts, it appeared as if the DDT in the feces actually was some of the DDT that had rubbed off the larvae. For this reason the external DDT shown in table 3 was calculated by combining the DDT recovered in the external larval rinses, cage and bean rinses, and fecal extracts.

The rate of absorption of DDT by these larvae was apparently quite slow; only 15% of the applied dose was absorbed in 120 hours. Part of this slow absorption, however, was due to the loss of DDT from the cuticle by abrasion.

Radiometric and colorimetric analyses of the internal larval extracts were almost identical. The amount of DDT inside the larvae remained essentially constant (0.7 or 0.8 μg. per larva) from 24 to 120 hours after treatment. The DDE inside the larvae increased from 0.1 μg./larva at 24 hours after treatment to 0.6 μg./larva at 120 hours. The absorbed DDT metabolized to DDE increased from 13% at 24 hours after treatment to 43% at 120 hours. No evidence of other metabolites was found. From 80 to 95% of the applied DDT was accounted for radiometrically. The unaccounted-for radioactivity was probably DDT in the holding cages and beans since the fecal and larval residues remaining after extraction contained negligible radioactivity.

Mortality of larvae during the experiment indicated that DDT affected the larvae quite slowly. Little emphasis could be placed on the value of 180 μg. of DDT/g. of body weight in these experiments because of the loss of DDT from the cuticle.

It was not practical to determine whether first instar larvae possess the same degree of tolerance to DDT as the fifth instar larvae. DDT has been used quite extensively to control this insect since 1946 and it is possible that some resistance to DDT has developed.

SUMMARY.—The absorption, metabolism, and excretion of *p,p'*-C^{14}-labeled DDT in adult, female Madeira roaches and fifth instar European corn borer larvae were studied.

The Madeira roach absorbed DDT rather slowly (about 90% in 20 days, fig. 1) and excreted 50% of the total applied radioactivity over a period of 36 days (fig. 1). Separation of the radioactive compounds excreted in feces by DDT-treated cockroaches was accomplished by paper chromatography. The presence of DDT, DDE, and three unidentified metabolites (fig. 3) was demonstrated. DDT was the predominant radioactive compound excreted the first 24 hours after treatment, after which a metabolite with an R_f value of about 0.88 was the major radioactive compound excreted. Metabolic pathways for DDT in the Madeira roach are proposed (fig. 4).

Fifth instar European corn borer larvae possess some tolerance to DDT and convert significant amounts of absorbed DDT to DDE (table 3). No evidence of DDT metabolites other than DDE was found. Both colorimetric and radiometric analyses were used in the corn borer study.

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Effect of Vapam on *Rhizoglyphus* Mites and Gladiolus Soil Diseases[1]

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Studies of the importance of mites of the family Acaridae have been in progress since experiments by Bald & Jefferson (1952) indicated that *Rhizoglyphus rhizophagus* Banks could be a serious primary pest of gladiolus. Other species which have been taken from gladiolus include *R. solani* Oudms. and *Caloglyphus spinitarsus* (Herm.).[4] *C. spinitarsus* is not common on gladiolus, and in greenhouse experiments caused no damage to healthy plants. *R. solani*, however, may be a pest of gladiolus, and Rawlins (1955) has shown that it was the cause of serious injury to onion seedlings in muckland soils in New York. Andison (1951) also reported that *Rhizoglyphus echinopus* (F. & R.) and *R. solani* have caused economic damage to the bulbs of certain varieties of narcissus and lily in British Columbia.

Collections during the last few years indicate that there is a complex of *Rhizoglyphus* mites occurring on gladiolus and other bulb crops in southern California. Mites collected during the course of this experiment were sent to E. W. Baker, who informed the writers that identification could not be made until this complex has been resolved. Symptoms of damage and conditions under which damage occurs, however, appear to be similar to those associated with infestation by *R. rhizophagus* and *R. solani*.

Rhizoglyphus mites are closely associated with gladiolus soil diseases, the most important of which are *Fusarium oxysporum* Schlecht f. *gladioli* Sn. and Hans., *Stromatinia gladioli* (Drayt.) Whet., and bacterial scab, *Pseudomonas*

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marginata (McCull.) Stapp. Damage from diseases and mites has been intensified in recent years as growers, due to the scarcity of new land, have been forced to plant a succession of crops in the same fields. Control involves two problems, the production of clean planting stock and the cleaning up of infested soil. The hot water treatment (Roistacher 1951, Bald & Markley 1955) will kill *Fusarium*, *Stromatinia* and the mites in cormels, but the temperature is too high for this treatment to be used on the corms. Ferguson & Markley (1955) showed that fumigation with methyl bromide or chloropicrin would control *Fusarium* and *Stromatinia* in the soil but the cost was very high.

In attempting to control *Rhizoglyphus* mites in the soil, a fumigant which did not also control the gladiolus diseases would be of no practical benefit. Vapam[5] was selected for trial because it appeared to be a general soil fumigant having fungicidal as well as insecticidal, nematicidal, and herbicidal properties. In a preliminary test, heavily infested corms were buried and the soil was treated with Vapam. Examination of the corms a week later indicated that the Vapam was effective against the mites and an experiment was set up to determine its effectiveness under field conditions against both the mites and soil-borne diseases on gladiolus.

METHODS.—The experiment was located in a field heavily infected with *Fusarium* and *Stromatinia* in which there had been several volunteer crops of gladiolus. Samples of volunteer corms were examined in August, 1954 and all were diseased and infested with mites. The Vapam was applied as single and split applications in separate but adjacent areas, using a randomized block design for each. In the single application the treatments were a check, 215, 430, and 860 pounds of Vapam per acre, and in the split application, a check and 430, 860, and 1720 pounds per acre. All treatments were replicated four times. The soil was prepared by disking and dragging. In the case of the split application the ground was so packed prior to the second application that it had to be reworked. This was done by disking in one direction only, to prevent dragging soil from the checks into the treated plots. Since the drag was not used the soil was uneven with many large clods, and the second application was not as good as the first. No estimate of reduced efficiency of the second application could be made; on the other hand, it was clear from the experimental results that it had a real effect.

The Vapam was applied with a Hozon proportioner and watered into the soil with Rainbird sprinklers which were run 2 hours. Plots receiving the split application were treated on September 8, 1954 and March 3, 1955, and those receiving the single application, on March 4, 1955. Since the main experiment was not to be planted until spring, an extra replicate, hereafter called the pilot planting, was also treated on September 8, in order to obtain some preliminary information on the effectiveness of the Vapam. Through an error at the time of the second application the treatments which had been used for the pilot planting were applied to those of the fourth replicate and consequently all data for the split application are from three replications only.

The pilot planting was planted on September 17, 1954. Two rows of Florence Nightingale no. 4 corms and one row of Phantom Beauty no. 4 corms were used in each plot. A sample of the Florence Nightingale corms planted in clean soil indicated that approximately 6% were infected with *Fusarium*. The corms from this planting were dug on March 3, 1955.

The main experiment was planted on March 17, 1955. The size of the plots was 16 by 25 feet and the rows were 19 feet long, leaving buffer zones between plots. In each plot there were two rows of Spotlight no. 3 corms, one row of Spotlight cormels, and two rows of Evangeline cormels. The corms of one Spotlight row and the cormels of one Evangeline row were dipped in New Improved Ceresan just before planting. While the cormels were planted according to normal practice, only 30 corms were used in each row. The cormels had received the hot water treatment and the Spotlight corms had been grown from hot-water treated cormels which had been planted in clean soil. Samples planted in the greenhouse indicated that approximately 5% of the Evangeline cormels, 1½% of the Spotlight cormels, and less than 5% of the Spotlight corms were infected with *Fusarium*. Both cormels and corms appeared to be free of *Stromatinia*, and no mites or indications of mite injury were found. There was a barely perceptible infestation of bacterial scab.

Observations on the growth of the plants in various treatments were made during the course of the experiment. Bloom counts were made on July 12, 1955, and at this time the number of plants missing, dead or severely diseased in the rows planted with Spotlight corms was recorded. Corms from all plots were dug on September 23, 1955. In determining the yields, all the corms in each row were weighed.

The mite counts were made by stripping off the mother corm and counting the mites in and around the basal plate. In the pilot planting the counts were made only on the Florence Nightingale, but on both clean and diseased corms. The 10 best-looking corms and 5 diseased corms of approximately the same size and general appearance were selected from each plot. In the main experiment the counts were made on the two best-looking corms from each row that had been planted with Spotlight corms.

Estimates of bacterial scab infection were obtained by means of a rating system using the following classification: 0, no infection; 1, mild infection but commercially acceptable; 2, severe infection; 3, mummified. Two blocks from the single and split applications provided replicates of 60 corms; 20 corms each from Spotlight cormels (undipped), the undipped Evangeline cormels and the dipped Evangeline cormels. Scab ratings were made only on corms grown from cormels, as yields from the Spotlight corms in some of the checks were too small for an adequate sample.

Estimates of the incidence of *Fusarium* disease were made from the same corms used in determining scab infection, and were based on the number of corms infected with *Fusarium*. Corms were not cultured and infection was determined from visible lesions, both external and internal. Single lesions varied from 2 or 3 mm. diameter to the size of the corm itself. Mummified corms without evidence of serious scab lesions were classified as *Fusar-*

[5] Sodium n-methyl dithiocarbamate dihydrate, furnished by Stauffer Chemical Company.

FIG. 1.—Effect of Vapam soil fumigation on growth of gladiolus: check plot in front, treated plots in rear; fourth replicate, single application, July 22, 1955.

ium. In the checks, there was so much loss from missing and premature disintegration of corms that consistent counts of *Fusarium* infection were not possible. The same series of corms gave counts of bacterial scab, only because corms mummified by scab did not disintegrate readily.

RESULTS.—*Yields*.—In both the pilot planting and the single application there were marked differences in growth between the plants in the checks and the treated plots within 2 months of planting, and by the time the plants came into bloom the checks were in very poor condition (Fig. 1). In the split application differences between checks and treated plots were less obvious, and did not appear until about the time the plants began to bloom.

Bloom counts (table 1) showed that dipping the corms in New Improved Ceresan before planting significantly increased the percentage of plants blooming in both the single and split applications. The Vapam treatments greatly increased the bloom in the single application but had little effect in the split application.

Bloom counts were not made in the pilot planting. However, the results were similar to those obtained in the single application of the main experiment. There were practically no blooms in the check plot and a high percentage of bloom in all the treated plots.

Table 1.—Percentage of plants blooming from dipped (New Improved Ceresan) and undipped Spotlight corms.

TOTAL VAPAM PER ACRE (POUNDS)	SINGLE APPLICATION		SPLIT APPLICATION	
	Dipped[a]	Undipped	Dipped[a]	Undipped
Check	33.1	20.8	69.0	45.3
215	68.6	54.4	—	—
430	84.0	73.6	76.1	49.5
860	94.0	83.9	74.2	68.2
1720	—	—	78.9	34.2

[a] Corms dipped in New Improved Ceresan before planting.

Table 2.—Yields of corms—mean weight in pounds.

TOTAL VAPAM PER ACRE (POUNDS)	SPOTLIGHT CORMS		SPOTLIGHT CORMELS		EVANGELINE CORMELS	
	Single Appl.	Split Appl.	Single Appl.	Split Appl.	Single Appl.	Split Appl.
Check	1.15	1.30	0.60	1.20	0.55	0.55
215	1.50	—	2.30	—	4.25	—
430	2.85	1.60	3.30	5.40	4.90	2.35
460	4.15	3.20	2.80	2.30	6.15	3.50
1720	—	3.65	—	3.00	—	4.40

Yields of corms from the various treatments are shown in table 2. All of the Vapam treatments significantly increased the yields from cormels, but from corms the increases were significant only at the two highest dosages in both the single and split application. Also, with one exception, yields were higher at the same dosages in the single application than in the split application. This may have been due partly to position in the field. Dipping the corms and cormels before planting in New Improved Ceresan did not significantly affect the yields from either corms or cormels, except for the Spotlight corms in the split application where the yield was increased.

In the pilot planting, all the Vapam treatments increased the yields of corms. The data from these treatments, shown in table 3, were not analyzed since the treatments were not replicated.

Mite counts.—The results of the mite counts on the harvested corms are summarized in table 4. For statistical analysis the square root transformation was used. For the combined data (dipped and undipped corms) analysis of variance indicated a significant reduction of the mites from all Vapam treatments in the single application and from the 860- and 1720-pound treatments in the split application.

Dipping the planting stock in New Improved Ceresan increased the variation within treatments and resulted in irregularities in treatment effects similar to those ob-

Table 3.—Yields of corms from pilot planting.

Total Vapam per Acre (Pounds)	Florence Nightingale		Phantom Beauty	
	Number of Corms	Mean Weight (Ounces)	Number of Corms	Mean Weight (Ounces)
Check	133	0.41	82	0.71
215	170	1.07	91	1.33
430	154	1.03	79	1.08
860	170	1.37	70	1.47

tained with bacterial scab (see later). There was a high value for the number of mites in the 860-pound treatment in the single application and the 430-pound treatment in the split application. These irregularities and the greater variation within treatments are believed to be due to the association of the mites with bacterial scab and not from any direct effect of the New Improved Ceresan.

Where the planting stock was not dipped there was a progressive reduction of the mites as the dosage of Vapam increased. Analysis of variance indicated significant differences between the checks and the Vapam treatments in the single application, and between the checks and the 860- and 1720-pound treatments in the split application. In the latter, the difference between the check and the 430-pound treatment barely lacked significance.

In the pilot planting the data were not analyzed statistically but they appeared to be a reliable indication of the effect of the Vapam treatments. The data indicated that the Vapam treatments reduced the mite population, and also that the mites were favored by disease.

Diseases.—In the pilot planting bacterial scab caused obvious infections in the check and the 215-pound treatment, but there were only traces at the higher dosages. In the main experiment, bacterial scab appeared in all treatments. The results are summarized in table 5.

There was a consistent fall in the bacterial scab ratings with increasing dosages for the corms from undipped Spotlight and Evangeline cormels, but for those from Evangeline cormels dipped in New Improved Ceresan, the scab ratings were very irregular. In the single application 215 pounds reduced the amount of scab, 430 pounds significantly increased it, and at 860 pounds the amount of scab was still high. In the split application, there was no test of the 215-pound dosage but results of the 430-, 860- and 1720-pound treatments were somewhat erratic.

The effect of the Vapam treatments on *Fusarium* is shown in table 6. Vapam reduced the incidence of *Fusarium* infection, probably at 215 pounds, and certainly at the higher dosages. At the 1720-pound dosage the percentage of corms infected did not differ significantly from that known to have been present in the planting stock. Differences between single and split applications of the same total dosage were not significant.

Table 4.—Average number of *Rhizoglyphus* mites per corm.

Total Vapam per Acre (Pounds)	Pilot Planting		Single Application		Split Application	
	Diseased Corms	Clean Corms	Dipped[a]	Undipped	Dipped[a]	Undipped
Check	56.6	12.2	264.3	175.0	107.0	108.0
215	27.6	4.5	146.0	50.3	—	—
430	29.2	8.3	22.8	32.3	163.0	53.0
860	18.0	4.6	101.8	24.8	38.3	45.0
1720	—	—	—	—	40.3	27.7

[a] Corms dipped in New Improved Ceresan before planting.

Table 5.—Mean ratings[a] for bacterial scab on corms grown from cormels.

Total Vapam per Acre (Pounds)	Single Application		Split Application	
	Dipped[b]	Undipped	Dipped[b]	Undipped
Check	1.45	1.40	1.80	1.75
215	0.65	1.10	—	—
430	1.70	1.00	1.20	0.88
860	1.20	0.68	0.40	0.70
1720	—	—	1.65	0.60

[a] 0 = no scab lesions, 1 = mild infection but commercially acceptable, 2 = severe infection, 3 = mummified.
[b] Corms dipped in New Improved Ceresan before planting.

The pilot planting indicated a reduction of *Stromatinia* by Vapam fumigation, but not elimination, even at the 860-pound dosage. In the main experiment there was no direct evidence of the effect of the Vapam treatments. Sampling of the corms after harvest failed to reveal the presence of *Stromatinia*, since temperatures during the summer were too high for the development of lesions on the daughter corms. However, *Stromatinia* was found in some of the root lesions during the earlier stages of growth, and was probably responsible for some missing and stunting of plants.

Discussion.—In this experiment which involved *Rhizoglyphus* mites and three diseases, the effect of the Vapam on the organisms was not measured directly but determined from the infection of corms grown in fumigated and unfumigated soil. Such a method, while showing the overall treatment effects on yields, often does not show clearly the effect of the fumigant on the individual organisms and makes evaluation of the results more difficult. This was illustrated in the mite counts, the bacterial scab ratings, and the fact that Stromatinia, while affecting the roots, did not produce visible lesions on the daughter corms in the main experiment.

In the pilot planting the mite counts were made on both clean and diseased corms. The latter showed obvious disease symptoms but were not rotting, and the corms selected for the counts were of approximately the same size and condition. In the main experiment the condition of the corms in the checks was such that similar counts could not be made accurately.

While there was a great deal of variation in the counts within treatments in the main experiment, this variation

Table 6.—Percentage of corms infected with *Fusarium*.

Total Vapam per Acre (Pounds)	Single Application	Split Application
Check	high[a]	high[a]
215	38.3	—
430	25.0	26.7
860	11.7	21.7
1720	—	2.5

[a] Not estimated.

was greater where the planting stock had been dipped in New Improved Ceresan. This is attributed to association of the mites with bacterial scab. Observations during the examination of the corms for both mites and disease indicated that the development of mite colonies was favored more by bacterial scab than *Fusarium*, and infection by bacterial scab was also quite erratic where the cormels had been dipped in New Improved Ceresan before planting.

In the pilot planting the mite counts were quite consistent. The greater number of mites per corm in the main experiment is attributed to the fact that this was a summer crop with soil temperatures more favorable to the development of the mites.

The reduction in the number of mites of corms from the Vapam treatments could be due to a reduction in the incidence of disease as well as to the direct effect of the Vapam on the mite population in the soil. However, in view of the results in the pilot planting where the effect of disease on the mites was eliminated as far as possible, it was concluded that the mite population in the soil was reduced by the Vapam treatments. Observations of a commercial trial also indicated that fumigation with Vapam reduced the mite population in the soil. Samples of corms from adjacent fumigated and unfumigated soil were examined. Corms from the fumigated soil had very few mites while those from the unfumigated soil were heavily infested and showed few disease symptoms.

Plants grown from hot water treated cormels have proved particularly susceptible to bacterial scab, probably for two reasons: 1) temperatures of 135°F. do not eliminate the scab organism from the cormels, and 2) the cormel microflora is greatly reduced. This microflora is assumed (Morrow *et al.* 1938, Bald 1947), by extension with the growing stems and roots, to supply much of the microflora which inhabits the rhizosphere and the surface of the underground stem of crop plants. It appears to be a natural reservoir for antagonists of such organisms as *Streptomyces scabies* (Thakt.) Waksman & Henrici, on potato, and *Psuedomonas marginata* (McCull.) Stapp on gladiolus.

Analysis of variance for bacterial scab in the Evangeline cormels in the single application illustrates the effect of the New Improved Ceresan. The variability of reaction of New Improved Ceresan-treated planting stock appears in a high value for intraplot variance, and there is a significant interaction between the New Improved Ceresan dip and two levels of Vapam dosage (check plus 430 pounds against 215 plus 860 pounds). The latter suggests that at higher dosages of Vapam the New Improved Ceresan encouraged the development of scab on the daughter corms. Presumably the reduction of microflora both on the corms and in the soil allowed infection of corms by relatively small quantities of inoculum. The variable figures for infection in the New Improved Ceresan series suggests erratic recolonization of the underground plant parts by antagonistic elements in the microflora. Since these results were obtained, a comparable effect of ethylmercury phosphate on the damping-off of pine seedlings (Gibson 1956) has been reported.

With respect to *Fusarium*, there was no evidence of irregularities in the effect of increasing dosages of Vapam, nor was there any significant effect on infection of daughter corms from dipping planting stock in New Improved Ceresan. The percentage of corms infected at the 1720-pound dosage did not differ significantly from that in the same lots of corms planted in steam-sterilized soil. This does not mean, however, that the 1720-pound dosage eliminated *Fusarium* from the soil. The incidence of infection on the test plants was based on lesions macroscopically visible on or in the tissue of the corm itself. Almost certainly, in all treatments, there were also infections which could only have been diagnosed by close examination and culturing.

SUMMARY AND CONCLUSIONS.—In soil heavily infested with *Rhizoglyphus* mites and three gladiolus soil diseases, fumigation with Vapam increased the yields of flowers and corms, reduced the mite infestation, and reduced infection from bacterial scab and *Fusarium* and probably from *Stromatinia*.

For the varieties tested, higher dosages were required for significant increases in corm yields from corm planting stock than from cormel planting stock. Dipping the planting stock in New Improved Ceresan resulted in irregularities in the effects of increasing dosages of Vapam on bacterial scab. New Improved Ceresan may upset the balance between a pathogen and antagonists derived from the planting stock and the soil. No such effect on *Fusarium* disease was observed.

Vapam appears to be a soil fumigant of moderate effectiveness against the gladiolus soil diseases, and the benefits to be obtained from its use will depend largely upon the degree of infestation of the soil and of conditions of climate and culture. In this experiment it was tested on very severely infested soil. Applied to less severely infested soils better results would be expected, and this has been demonstrated in commercial trials. From the standpoint of costs and increased production, dosages of 300 to 400 pounds per acre appear to be economically practical.

A great deal still remains to be learned about Vapam, including the best methods of application under various conditions, the cumulative effects of successive applications, and its effect on the soil microflora. It has already been found in both experimental work (Ferguson & Markley 1955) and growers' plantings, that untreated planting stock in fumigated soil can result in an increase rather than a reduction of disease.

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Studies on the Mechanics of Feeding of the Spotted Alfalfa Aphid on Alfalfa[1]

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Studies of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), (also called *Pterocallidium* sp.) have been of considerable interest since the first report of damage to alfalfa in the United States in February of 1954. From its appearance in New Mexico, the insect has spread over the southwestern states of Arizona, Texas, and Oklahoma. In the west, California, Colorado, Nevada, and Utah have become infested. The infestation has moved northward into Kansas and Missouri, eastward into Arkansas, Louisiana and Mississippi. An isolated infestation in Florida has recently been reported (Anonymous 1956). Losses to growers of alfalfa have been large and the need for control of the insect has become of great economic importance.

Dickson *et al.* (1955) have reported on the life history and population trends in California. These investigators state that the plant damage is the result of sucking the juice from the plant by the insects and at the same time injecting saliva which may, or may not, be toxic.

Bieberdorf & Bryan (1956) have recently reported on control measures being taken against this aphid in Oklahoma.

The study of Chatters & Schlehuber (1951) on the feeding mechanics of the greenbug (*Toxoptera graminum* Rond.), another aphid, showed that insect to be primarily a phloem feeder. Additional damage resulted from removal of the contents of parenchyma cells and the lysis of parenchyma by salivary secretions. A specific investigation of the methods of feeding of the spotted alfalfa aphid has not been reported. For this reason this problem was undertaken by the writers.

MATERIALS AND METHODS.—Alfalfa infested with the insects was available in the fields near Stillwater, Oklahoma, and was in all instances used for the present study. Although potted greenhouse plants were infested, the internal anatomy of the leaves of these was radically different from that of the field-grown specimens. Use of the leaves of greenhouse plants as control or comparison materials was not considered to be wise.

Normal histological techniques were employed except in killing and fixing of the leaves bearing attached insects.

Since field materials were employed, it was believed essential to have specimens with the stylet inserted to insure that damage relationships were not confused with those of other sources. Original winter collections, killed in FAA[4] were satisfactory. Later attempts to secure attached insects by this means failed as the insects withdrew their stylets before death. Other killing agents, anesthesia, and refrigeration of infested plants prior to killing likewise failed. Good results were eventually obtained by killing the plant parts with the attached insects by sudden immersion in FAA which had been previously cooled to 0° C.

Dehydration was accomplished by means of the normal butyl-ethyl alcohol and tertiary butyl-ethyl alcohol procedures. Sections were stained with safranin 0-fast green FCF, Mayer's modified hemalum-safranin 0, and a modification of the Triarch quadruple stain.

MODE OF PENETRATION.—The stylets of the spotted alfalfa aphid were found to be inserted intercellularly through the epidermis and mesophyll parenchyma. Salivary stylet sheaths were formed about the stylets and these structures stained vividly with safranin 0. Stomatal penetration was not observed.

FEEDING SITES.—Feeding sites of the spotted alfalfa aphid appear to be the mesophyll parenchyma and the phloem of vascular bundles. These sites are fundamentally similar to those of the greenbug (Chatters & Schlehuber 1951). A difference in the degree of selectivity of feeding areas appeared to exist between the two insects, however. The spotted alfalfa aphid, although terminating the vascular bundle punctures within the phloem, appeared to make considerably more parenchyma terminations of the stylets. Chatters & Schlehuber (1951) found the greenbug highly selective in the seeking of phloem tissue.

VASCULAR FEEDING.—The stylets with their sheaths were found to follow an intercellular course which, although meandering somewhat, terminated in the phloem. Penetration of the bundle was most commonly from the dorsal (adaxial) side and necessitated an intercellular traversing of the xylem (figures 1 and 2). At this point diffusion of the salivary substance produced what appeared as an exaggeration of secondary wall thickening in the primary xylem. This diffusion of the secreted material was observed for a distance of from 30 to 40μ on either side of the stylets. The course of the stylets was, in many

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[4] Formaldehyde (40%) 10 ml., acetic acid (glacial) 5 ml., ethyl alcohol 50 ml., and water 35 ml.

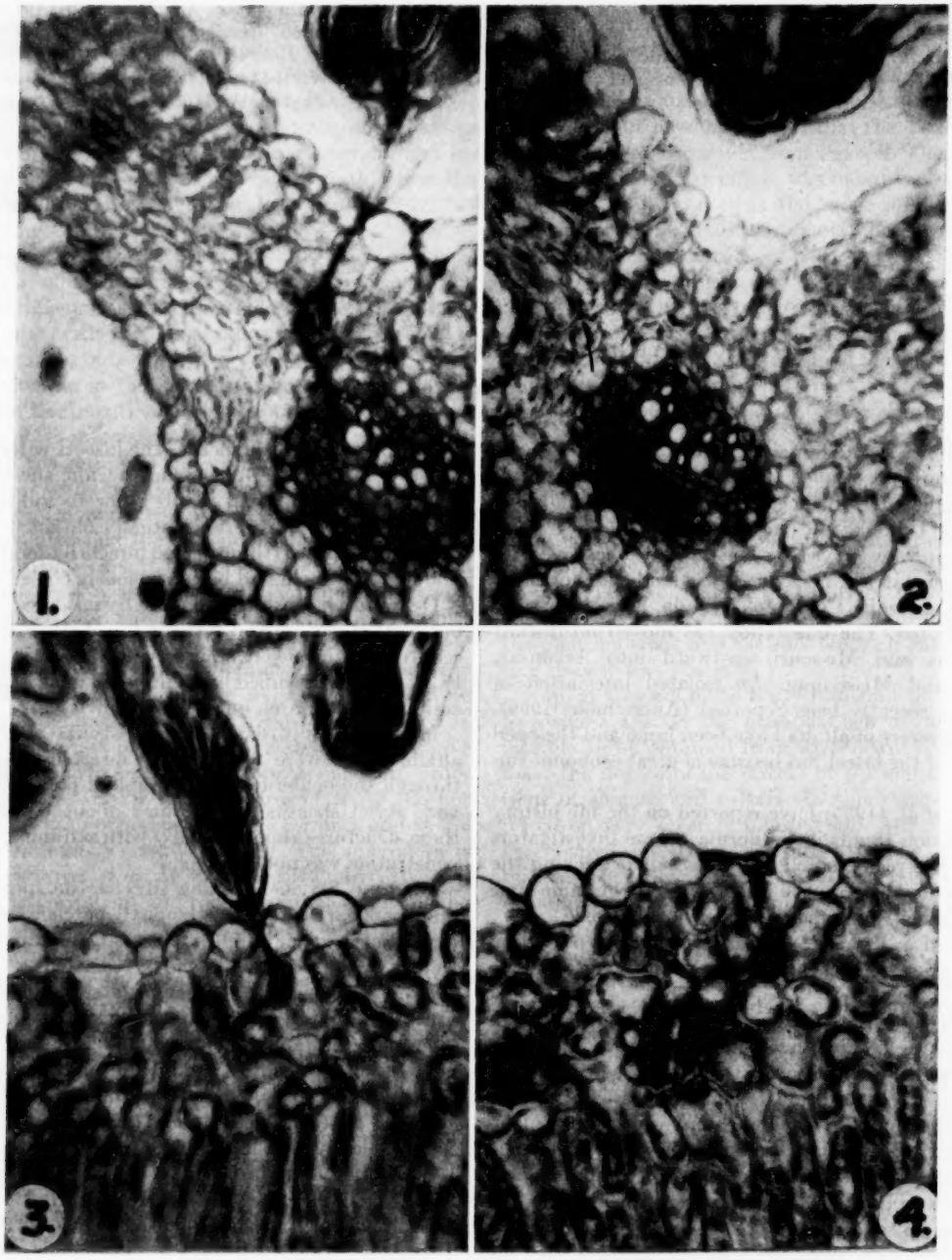

FIG. 1.—Penetration of stylets into midrib vascular bundle to a position in the xylem. Note stained secretion about nearby xylem. (×360)

FIG. 2.—Same as above but deeper section of same specimen showing termination of a stylet in the phloem by acute bending. (×360)

FIG. 3.—Intercellular path of the stylets toward a small vascular bundle. (×360)

FIG. 4.—Diffused insect secretion and group of parenchyma cells with contents undergoing lysis or being obscured. (×360)

instances, apparent only upon studying serial sections. Figures 1 and 2 are photomicrographs of two sections of the same puncture. In fig. 2 is shown the terminal portion of a stylet which penetrated the phloem at an acute angle and approximately 20μ removed from the preceding portion.

No attempt was made to determine whether vascular feeding resulted in the actual withdrawal of plant fluids, but it is presumed to occur.

PARENCHYMA FEEDING.—As mentioned previously, the spotted alfalfa aphid appears to do a considerable portion of its feeding in the parenchyma. Some of the damaged areas may be the result of unsuccessful penetrations toward vascular tissues, hence, any quantitative estimation

of the two feeding patterns is not feasible with this limited study.

When the stylets terminated in the parenchyma the site was usually conspicuous by the large darkly stained area with diffuse boundaries. See fig. 4. Many such areas were found in the parenchyma of infested leaves.

It should be noted that the stainable material was not confined to the stylet sheaths or the plant cell walls but was diffuse within the cell. Chloroplasts and nuclei have been obscured. In brief, such areas usually contained cells in which the contents appeared almost homogeneous. Upon macroscopic examination, the infested leaves were seen to bear tiny chlorotic spots. It would appear reasonable that such spots were the result of destruction of the chloroplasts.

The diffuse distribution of the injected substance, the chlorotic spotting and the absence of chloroplasts in microscopic section indicate that the inserted contaminant is highly toxic and has a plasmolytic effect.

Neither conspicious lysis of cell walls nor the presence of large craters as produced by the greenbug (Chatters & Schlehuber 1951) in barley was observed with this aphid. This might suggest that the injected secretion of the spotted alfalfa aphid is more specific for cytoplasmic materials than is the case with the greenbug.

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SUMMARY.—Alfalfa leaves which were infested with spotted alfalfa aphids were examined by histological and cytological methods. Results show that the insect inserts its stylets intercellularly and feeds within the phloem and mesophyll parenchyma. Considerable quantities of saliva or other toxic substances are injected into the tissues.

Greatest amounts of damage are evident within the mesophyll where the toxic substance or substances appear to cause lysis of the cytoplasm. These areas may be visibly correlated with the macroscopic chlorotic areas of the leaves.

Removal of manufactured foods from the plant by phloem feeding probably occurs and would doubtlessly contribute to weakening of the host. The determination of the contribution of each of the feeding methods, to the overall damage to the alfalfa plant was not attempted during this investigation.

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Effects of Piperonyl Butoxide on the Anticholinesterase Activities of Some Organic Phosphorus Insecticides on House Fly and Purified Bovine Erythrocyte Cholinesterases[1]

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The anticholinesterase activity of the organic phosphorus insecticides is important in their insecticidal action. This view has been strengthened by the recent demonstration of the presence of acetylcholine in *Calliphora* and *Lucilia* (Lewis 1953). Results by Rai *et al.* (1956), on DDT-susceptible and resistant house flies by topical application, suggest that the presence of piperonyl butoxide decreased the toxic actions of malathion on both strains but markedly synergised the lethal effects of Diazinon and Bayer L 13/59.

Since a cholinesterase system in some way is involved in the toxic action of malathion, Diazinon, and Bayer L 13/59 their effects with piperonyl butoxide on cholinesterase (ChE) obtained from house fly brain breis and on a purified bovine erythrocyte ChE were investigated.

METHODS AND MATERIALS.—The colorimetric method of Hestrin (1949) for the quantitative determination of acetylcholine was used to measure per cent inhibition of ChE.

House fly brei was prepared from the heads of 4-day-old male *Musca domestica* L. reared according to National Association of Insecticide and Disinfectant Manufacturers procedures at 80° ± 2° F. Buffer solution containing 0.15 M sodium chloride, 0.04 M magnesium chloride, and 0.025 M sodium bicarbonate was employed, at 1 ml. for each head, for making the ChE preparation. The pH of buffer varied from 7.68 to 7.72.

To 20,000 units of purified bovine erythrocytes acetyl cholinesterase (AChE)[3] in a sealed bottle, 10 ml. of ice-cold sterile saline solution (0.9% weight/volume) was added by puncturing the rubber seal with a syringe. This

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stock solution of AChE was diluted to keep the enzymatic hydrolysis rate of acetylcholine, 2.5 μM in a 30-minute reaction period, within the comparable range of enzymatic activity obtained from the fly ChE. This final standard test solution of AChE was refrigerated except for brief periods to withdraw test quantities. Acetylcholine bromide, 0.05M, in 0.001N sodium acetate was the substrate in all tests.

Fresh solutions of inhibitors or inhibitor-piperonyl butoxide were made up in glass distilled acetone for each test. The reaction flasks were 22×90 mm. shell vials, to each of which 2.6 ml. of buffer solution containing ChE and 0.1 ml. of inhibitor preparation was added. For each test a non-enzymatic flask was used containing only 2.6 ml. of buffer and 0.1 ml. of acetone, and two enzymatic flasks were employed which contained only 2.6 ml. of buffer containing ChE and 0.1 ml. of acetone. The reaction flasks were shaken well and immersed in a Warburg water bath at 36.5° ± 0.02° C. Fifteen minutes after addition of the inhibitors, 0.3 ml. of the substrate was introduced into the flask. At the end of a 30-minute reaction period, three 1-ml. aliquots were taken from each flask and the color developed. One aliquot was employed for the nonspecific color control to serve as a blank, and the other two served as replicates. Readings were taken at 540 mμ with Bausch and Lomb "Spectronic 20" within 10 minutes after the color development.

Where the ChE preparations were made from piperonyl butoxide treated flies, the flies had been topically treated with a 10 times more concentrated (weight/volume) piperonyl butoxide solution than the highest concentration of malathion used in the test. Brain brei ChE was prepared as described above after the desired time interval.

RESULTS AND DISCUSSION.—Table 1 shows the per cent inhibition of ChE from different sources with malathion and malathion-piperonyl butoxide mixtures at four concentrations of malathion in three paired tests. The presence of piperonyl butoxide decreased the anticholinesterase activities of malathion on all except four ChE samples.

The analysis of variance of per cent inhibition of brain brei ChE from the DDT-susceptible KUN strain with malathion and with malathion-piperonyl butoxide (table 2) shows a difference between the two treatments significant at the 1% level. The differences between the different dosages (molar concentrations) were significant at the 0.1% level. Except with the first molar concentration, with KUN-ChE there was a significant difference between inhibition of ChE caused by malathion and by malathion-piperonyl butoxide (table 3).

Essentially the same type of conclusions can be drawn for the DDT-resistant Campus strain-ChE (table 2). The difference between the two treatments (malathion and malathion-piperonyl butoxide) was significant at the 1% level. All differences in effect of concentrations of malathion tested for Campus-ChE (tables 2 and 4) were significant at the 0.1% level.

For the per cent inhibition of ChE, from both strains, with malathion and/or malathion-piperonyl butoxide

Table 1.—Inhibition of house fly brain brei ChE and purified AChE by malathion (M), and malathion plus piperonyl butoxide (MPB), 1:10 ratio by weight.

Source of ChE	Molar Concentration of Malathion	Per Cent Inhibition					
		Test 1		Test 2		Test 3	
		M	MPB	M	MPB	M	MPB
Brain brei ChE of KUN[a]	1.01×10^{-4}	88	88	88	75	88	87
	5.05×10^{-5}	75	54	67	59	62	65
	2.53×10^{-5}	62	46	56	42	42	44
	1.27×10^{-5}	21	17	38	25	25	14
Brain brei ChE of Campus[b]	1.01×10^{-4}	88	85	92	71	90	86
	5.05×10^{-5}	65	58	67	62	72	73
	2.53×10^{-5}	52	42	54	42	47	46
	1.27×10^{-5}	35	31	34	25	29	27
Purified AChE[c]	1.50×10^{-3}	82	42	90	52	91	52
	7.50×10^{-4}	53	24	74	38	62	38
	3.75×10^{-4}	35	12	37	24	34	24
	1.88×10^{-4}	6	0	16	10	24	10

[a] DDT-susceptible strain of house fly.
[b] DDT-resistant strain of house fly.
[c] Bovine erythrocytes ChE.

Table 2.—Analysis of variance of per cent inhibition of ChE from various sources with malathion and malathian plus piperonyl butoxide, 1:10 ratio by weight.

Sources of ChE	Sources of Variance	df	Mean Square
KUN[a]	Between treatments	1	532.05**
	Dosages	3	4399.60***
	Replicates	2	4.90 n.s.
	Treatment×dosages	3	20.48 n.s.
	Error	14	45.31
	Total	23	
Campus[b]	Between treatments	1	247.04**
	Dosages	3	3406.70***
	Replicates	2	16.79 n.s.
	Treatment×dosages	3	9.82 n.s.
	Error	14	24.13
	Total	23	
Purified[c] AchE	Between treatments	1	3220.10***
	Dosages	3	3692.80***
	Replicates	2	73.80
	Treatment×dosage	3	283.40***
	Error	14	7.45
	Total	23	

[a] DDT-susceptible strain of house fly.
[b] DDT-resistant strain of house fly.
[c] Purified bovine erythrocytes ChE.
** Significant at 1 per cent level.
*** Significant at 0.1 per cent level.
n.s. Non-significant.

Table 3.—Descending array of mean per cent inhibition of brain brei ChE from KUN[a] strain with malathion (M) and malathion plus piperonyl butoxide (MP), 1:10 ratio by weight.

Molar Concentration of Malathion	Mean Per Cent Inhibition		
	M		MBP
1.01×10^{-5}	88	n.s.	83.3
5.05×10^{-5}	68.0	*	59.3
2.53×10^{-5}	53.3	*	42.3
1.27×10^{-5}	28.0	*	14.6

L.S.D. = 6.31 at 0.05 level.

[a] DDT-susceptible strain.
n.s. = Non-significant difference.
* Difference significant at 5% level.

Table 4.—Descending array of mean per cent inhibition of brain brei ChE from Campus[a] strain with malathion (M) and malathion plus piperonyl butoxide (MP), 1:10 ratio by weight.

Molar Concentration of Malathion	Mean Per Cent Inhibition		
	M		MPB
1.01×10^{-5}	90.0	*	80.6
5.05×10^{-5}	68.0	n.s.	64.6
2.53×10^{-5}	51.0	*	43.3
1.27×10^{-5}	32.6	*	27.6

L.S.D. = 4.61 at 0.05 level.

[a] DDT-resistant strain.
n.s. = Non-significant difference.
* Difference significant at 5% level.

(table 2), there was no significant difference in the replicates (tests 1, 2 and 3), nor in treatment-dosage interaction.

No difference was found in the ChE contents of the brain breis of KUN and Campus strains of house flies. These similarities in ChE activity of DDT-susceptible and resistant strains of house flies have been pointed out by March (1952) and Pratt & Babers (1953). Responses of cholinesterase from both strains of house flies to malathion and malathion-piperonyl butoxide were almost the same.

Analysis of variance of per cent inhibition of purified AChE with malathion and malathion-piperonyl butoxide (table 2) indicate significant difference at the 0.1% level. This difference is further illustrated in descending array of mean per cent inhibition of purified AChE with malathion and malathion-piperonyl butoxide combinations (table 5).

The per cent inhibition of purified AChE with malathion and piperonyl butoxide-malathion, at various ratios, is presented in table 6. Four separate tests were run. In each test, fresh solutions of malathion and malathion-piperonyl butoxide were tested with one non-enzymatic and two enzymatic control samples.

The analysis of variance (table 7) shows (at 0.1%) significant difference in the effect of the concentrations of malathion, a fact already discussed in table 2. The differences in the level of inhibition of ChE with malathion in combination with varying ratios of piperonyl butoxide were significant at the 5% level. A significant difference in the response of ChE malathion can be noticed (table 7) which may be accounted for by slight differences in the preparations of inhibitor solutions for different tests.

Table 5.—Descending array of mean per cent inhibition of purified AChE[a] with malathion (M) and malathion plus piperonyl butoxide (MP), 1:10 ratio by weight.

Molar Concentration of Malathion	Mean Per Cent Inhibition		
	M		MPB
1.50×10^{-3}	87.6	*	48.6
7.50×10^{-4}	63.0	*	33.3
3.75×10^{-4}	35.3	*	20.0
1.88×10^{-4}	15.3	*	6.3

L.S.D. = 2.53 at 0.05 level.

[a] Bovine erythrocytes ChE.
* Difference significant at 5% level.

Table 6.—Effects of different ratios of piperonyl butoxide (PB) to malathion (M) on the anticholinesterase activities of malathion on purified bovine erythrocytes ChE.[a]

Ratio of P.B. to M	Molar Concentration of M	Per Cent Inhibitions							
		Test 1		Test 2		Test 3		Test 4	
		M	MPB	M	MPB	M	MPB	M	MPB
5:1	1.50×10^{-3}	76.9	50.0	85.7	57.1	75.0	41.7	87.0	60.9
	7.50×10^{-4}	53.8	34.6	52.4	42.9	50.0	33.3	60.9	43.5
	3.75×10^{-4}	26.9	23.1	42.9	28.6	29.6	20.8	47.8	30.4
	1.88×10^{-4}	15.4	11.5	23.8	14.3	12.5	8.3	13.1	8.7
10:1	1.50×10^{-3}	79.3	47.0	75.4	57.0	88.0	41.0	75.0	46.0
	7.50×10^{-4}	64.3	30.0	60.0	47.0	48.0	26.0	58.0	33.0
	3.75×10^{-4}	51.8	21.0	48.0	39.0	31.0	15.0	35.0	25.0
	1.88×10^{-4}	18.0	10.0	43.0	32.0	19.0	11.0	21.0	17.0
20:1	1.50×10^{-3}	84.2	42.1	63.6	40.9	86.4	50.0	85.7	47.6
	7.50×10^{-4}	47.4	26.3	45.5	27.3	63.6	36.0	61.9	33.3
	3.75×10^{-4}	36.9	15.6	31.8	18.2	36.4	27.3	33.3	19.0
	1.88×10^{-4}	13.2	10.5	13.6	4.5	22.7	18.2	9.5	4.8

[a] In each test parallel samples for malathion alone (M) and malathion plus piperonyl butoxide (MPB) at various ratios were run with a common enzymatic and non-enzymatic control for the substrate.

Since the same concentration of malathion gave varying levels of inhibition (table 6), the proper evaluations as to which ratio of piperonyl butoxide produced the least inhibition (or maximum antagonistic action) of AChE with malathion was difficult to obtain from the per cent inhibition figures. To obtain this information, all the per cent inhibition figures for malathion-piperonyl butoxide (table 6) were plotted against molar concentrations on logarithmetic-probit paper, and In_{50} values were estimated by extrapolation. In_{50} values of malathion in combination with varying ratios of piperonyl butoxide are presented in table 8. The mean In_{50} concentrations of malathion at 5:1, 10:1, and 20:1 ratios (Piperonyl butoxide to malathion) are 1.29×10^{-3} M, and 1.17×10^{-3} M, and 1.92×10^{-3} M, respectively. The higher ratios of piperonyl butoxide tend to decrease the anticholinesterase activities of malathion.

To study the effect of piperonyl butoxide on the ChE of the house fly, the KUN strain flies were topically treated on the mesosternum with piperonyl butoxide solution in acetone. After varying time intervals the flies were sacrificed and brei ChE was prepared. Flies treated with acetone were used for controls. On these two ChE preparations, the effectiveness of the malathion was tested. In three replicates on a single batch of flies 16 hours after treatment with piperonyl butoxide, 76 and

Table 7.—Analysis of variance of anticholinesterase activities of malathion in combination with varying ratios of piperonyl butoxide on purified bovine erythrocytes ChE.

Sources of Variance	df	Mean Square
Dosages	3	2818.42***
Tests[a]	3	135.69*
Ratios	2	141.75*
Dosage×ratio	6	29.25 n.s.
Error	33	39.60

[a] Variance was only analyzed on the malathion plus piperonyl butoxide treatment, for which the per cent inhibition figures are presented in table 9.
* Significant at 5% level.
*** Significant at 0.1% level.
n.s. = Non-significant.

Table 8.—In_{50} values of malathion used with varying ratios of piperonyl butoxide for purified cholinesterase.[a]

Test No.	Ratio of Piperonyl Butoxide to Malathion		
	5:1	10:1	20:1
1	1.50×10^{-3}	1.80×10^{-3}	2.20×10^{-5}
2	1.10×10^{-3}	0.95×10^{-3}	2.20×10^{-3}
3	1.60×10^{-3}	2.30×10^{-3}	1.60×10^{-3}
4	0.95×10^{-3}	1.80×10^{-3}	1.70×10^{-3}
Total	5.15×10^{-3}	6.85×10^{-3}	7.70×10^{-3}
Mean In_{50} concentration of malathion	1.29×10^{-3}	1.71×10^{-3}	1.92×10^{-3}

[a] In values have been derived by plotting the probit transformation of per cent inhibition values (table 9) on fitted regression lines

75% inhibitions were observed in acetone and piperonyl butoxide-treated flies, respectively, with 5.05×10^{-5} M malathion. This suggests that, under *in vivo* conditions, the antagonistic or protective effect of piperonyl butoxide against the action of malathion on ChE system of flies is practically non-existent. In another test the inhibitions by malathion of ChE from the KUN strain were found to be 67 and 64%, respectively, for acetone and piperonyl butoxide-treated flies 1 hour after treatment. The protective effect of piperonyl butoxide against malathion inhibition to ChE under *in vivo* conditions at 1 hour was more than at 16 hours.

On the assumption that piperonyl butoxide does protect the ChE system (under *in vivo* conditions in the house fly) against the anticholinesterase activities of malathion, tests were run on flies of three different batches and ChE preparations were made 6 hours after the treatment with piperonyl butoxide and acetone. The test was replicated three times. The per cent inhibition of ChE with 5.05×10^{-5} M is presented in table 9. Analysis of variance

Table 9.—Inhibiting action of malathion (5.05×10^{-5} M) on ChE obtained from the KUN flies after 6 hours of the treatment with piperonyl butoxide.[b]

Batch[c] of Flies	Replication	Per Cent Inhibition	
		ChE-from Acetone-treated Flies[a]	ChE-from Piperonyl Butoxide-treated Flies
A	1	63.3	61.2
	2	63.3	61.2
	3	63.3	61.2
B	1	67.9	64.7
	2	67.9	64.7
	3	67.3	64.7
C	1	69.2	65.5
	2	69.2	65.5
	3	69.2	65.6

[a] Same number of flies from the same batch were treated with acetone to serve as a source of ChE for testing the effect of malathion on PB-treated flies.
[b] Concentration of piperonyl butoxide in acetone was 10 times more (weight/weight) than the concentration of malathion solution.
[c] Each batch represents flies of the same age obtained on 3 different days.

Table 10.—Analysis of variance of mean per cent inhibition of ChE, obtained from KUN flies for each batch after 6 hours of the treatment with piperonyl butoxide.

Source of Variance	df	Mean Square
Batches	2	14.312*
Treatments	1	12.9066*
Error	2	0.3217

* Significant at 5% level.

tested on the mean per cent inhibition figures is presented in table 10. There was a significant difference (5% level) in the amount of inhibition of ChE from flies treated with acetone and with piperonyl butoxide. Also, the differences among different batches were significant at the 5% level.

Unpublished results from this laboratory on the effectiveness of piperonyl butoxide on the lethal action of Diazinon and Bayer L 13/59 on DDT-susceptible and resistant strain of house flies and the results of Hoffman *et al.* (1954) with Diazinon-piperonyl butoxide on a DDT-resistant strain of house fly strongly suggest a synergistic effect of piperonyl butoxide on the lethal action of organic phosphorus compounds. The synergistic effects of piperonyl butoxide on the action of Diazinon and Bayer L 13/59 with the ChE system of flies was investigated. Results of *in vitro* inhibition of ChE from different sources with Diazinon and Bayer L 13/59 are presented in table 11. Three separate paired tests (inhibitor and inhibitor-piperonyl butoxide) were run on different batches of fly ChE. No difference was found in the per cent inhibition levels produced by Diazinon and Bayer L 13/59 when used alone or in combinations with piperonyl butoxide.

Table 11.—*In vitro* inhibition of ChE and purified AChE by Diazinon and Bayer L 13/59, without piperonyl butoxide.

Source of ChE	Inhibitors	Molar Concentrations	Per Cent Inhibition[a]	
			Toxicant	Toxicant +PB[b]
Brain brei ChE of KUN[c]	Diazinon	7.33×10^{-5}	79	79
		3.66×10^{-5}	45	52
		1.83×10^{-5}	24	28
		9.15×10^{-9}	17	17
	Bayer L 13/59	6.50×10^{-5}	83	83
		3.25×10^{-5}	59	59
		1.62×10^{-5}	38	38
		8.10×10^{-9}	22	28
Brain brei CHE of Campus[d]	Diazinon	7.33×10^{-5}	84	82
		3.66×10^{-5}	60	52
		1.83×10^{-5}	33	27
		9.15×10^{-9}	14	11
	Bayer L 13/59	6.50×10^{-5}	70	70
		3.25×10^{-5}	45	45
		1.62×10^{-5}	25	28
		8.10×10^{-9}	12	12
Purified AChE[e]	Diazinon	8.25×10^{-6}	95	95
		1.65×10^{-6}	62	62
		1.10×10^{-6}	39	39
		5.50×10^{-7}	22	22
	Bayer L 13/59	3.90×10^{-5}	65	65
		1.95×10^{-5}	42	42
		9.75×10^{-6}	18	18
		1.95×10^{-6}	6	6

[a] Per cent inhibition values are based on three sets of paired tests with two treatments having a common enzymatic control.
[b] Piperonyl butoxide. Ratio of malathion to P.B. was 1:10 by weight.
[c] DDT-susceptible strain.
[d] DDT-resistant strain.
[e] Bovine erythrocytes ChE.

SUMMARY AND CONCLUSIONS.—The effects of piperonyl butoxide on the anticholinesterase activities of malathion, Diazinon, and Bayer L 13/59 on ChE, from a DDT-susceptible and a resistant strain of house flies and purified bovine erythrocyte AChE were investigated *in vitro* by colorimetric means. A significant decrease in ChE inhibition was found wherever malathion was employed in combination with piperonyl butoxide. This lower anticholinesterase action of malathion, due to the presence of piperonyl butoxide, was more pronounced on purified AChE than it was on house fly ChE. The decreased inhibition of purified AChE was associated with the higher ratios of piperonyl butoxide.

The *in vivo* protective effects of piperonyl butoxide to fly ChE against the inhibitory effects of malathion were investigated at varying intervals after the topical applications of piperonyl butoxide solution in acetone to individual flies. The results 6 hours after treatment suggest that piperonyl butoxide protects the ChE against the inhibitory actions of malathion. No protective effect was found 16 hours after the application of piperonyl butoxide.

Diazinon and Bayer L 13/59, in combination with piperonyl butoxide gave synergistic toxic effects by topical applications. No indications of anti-ChE inhibition was observed in the *in vitro* tests.

Contrasting effects of piperonyl butoxide on different organic phosphorus insecticides suggests differences in the mode of action.

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Observations on Some Filth Flies in the Vicinity of Fort Churchill, Manitoba, Canada, 1953–54[1]

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In the course of studies conducted to determine the species of insects which may be of actual or potential importance in the transmission of human enteric diseases in arctic and sub-arctic areas, considerable information was obtained as to species[3] of flies attracted to filth, their seasonal occurrence, biology and habits, in the vicinity of Fort Churchill. This paper presents certain aspects of this information.

GEOGRAPHY AND CLIMATE.—Fort Churchill is located on the west shore of the Hudson Bay at the mouth of the Churchill River, at longitude 94° 17′ W. and latitude 58° 47′ N. The normal mean temperature records of the region for the past 20 years show 227 days with temperatures below freezing; there are only 2 months of the year during which freezing does not occur. There are 122 days of the year with temperatures below 0° F. The mean annual temperature is 17.8° F. July and August are the warmest months with means of 53.7° and 52.4° F. respectively. January is the coldest month with an average temperature of −19° F. Precipitation in the Churchill area is light throughout the year and occurs mostly in the spring and summer when the ground remains wet and soggy. This soggy condition is due in part to the permafrost near the surface, slow evaporation and the thick layer of moss and spongy vegetation. The mean annual snowfall is 57 inches. The annual precipitation is 10.27 inches. Poor drainage of the permafrost area results in the formation of countless pools of stagnant water. During July and August the myriads of swampy areas and streams serve as breeding places for countless mosquitoes, black flies and tabanids.

FILTH FLIES.[4]—There is a scarcity of published information on the occurrence and habits of filth flies in the sub-arctic and arctic regions. Several species of flies attracted to human feces during bacteriological and waste disposal studies in the Churchill area in 1951 and 1952 prompted the instigation of this entomological study.

MATERIALS AND METHODS.—Ordinary conical type

[1] Studies made upon the recommendation of the Subcommittee on Waste Disposal, Committee on Sanitary Engineering and Environment, National Research Council and sponsored by The Army Environmental Health Laboratory as a Class II activity under the Army Surgeon General. Some of this information has appeared in a special report by that laboratory. The Defense Research Northern Laboratory of the Canadian Defense Research Board served as headquarters for the project. Accepted for publication Feburary 7, 1956.

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[3] Identification made by members of the Entomology Research Branch, U. S. Dept. of Agriculture, Washington, D. C.

[4] In this paper the term "filth flies" is used to signify their association with human waste, and also to indicate the exclusion of "biting flies."

fly traps baited with various attractive materials were operated throughout the season of fly activity to determine the species attracted to these baits and to attempt to evaluate their relative potential importance as carriers of human pathogens. Observations and hand collections of flies were also made in army messes, in restaurants in the town of Churchill, at garbage disposal sites, and at winter and summer troop bivouac sites. Immature stages were collected and reared from naturally occurring breeding media when possible. Some observations were also made on overwintering stages in nature.

GENERAL INFORMATION.—Observations in the Fort Churchill area in 1953 extended from June 5 to October 10 and in 1954 from May 5 to September 21. Several species of flies were active at the time of the earliest observations both years, but extensive adult fly activity did not occur either year until about mid-July. Activity was very high for the latter part of July and the first half of August, but it dropped sharply in late August and remained light during September. The only species observed active in the adult stage out-of-doors as late as October was *Cynomyopsis cadaverina*.

Filth flies were not a nuisance or health problem in summer bivouacs, fishing camps, or other outdoor activities. At the post garbage disposal area located about one-half mile from Fort Churchill, two species of Calliphorids (*C. cadaverina* and *Protophormia terraenovae*) occurred in enormous numbers, and *Musca domestica* was found in considerable numbers in late July and August. None of these species ever occurred in noticeable numbers in the homes or around garbage stands at homes or messes located less than 1 mile from the post dump. A few of each of these three species were taken inside the two post messes but were not numerous enough to be a problem. Several miles from known breeding areas, large numbers of the two Calliphorids were collected in traps that were baited with human feces or with cooked meats. This however was not true with *M. domestica*, since only one specimen was collected in a trap baited with human feces located at a winter bivouac site 5 miles from Fort Churchill.

Post Garbage Dump as a Source of Flies.—Early in June 1953, adults of *C. cadaverina* and *P. terrae-novae* were seen ovipositing on the carcass of a dog at the post garbage dump. Later in June, these species were laying eggs on meat scraps and other exposed garbage. In late July and in August both species were extremely numerous, particularly *P. terrae-novae* which appeared to reach its peak of adult abundance about the last week in July. It remained the most prevalent species in August. *Hydrotaea bispinosa* appeared the first week in July and became the third most abundant species in late July. *Paregle radicum* and *Paregle mystacea* were both present in considerable numbers at the dump throughout July. *Muscina assimilis* was observed in small numbers in late June and early July. *Musca domestica* was first observed at the dump on 3 August and became fairly abundant by late August.

FLY TRAP COLLECTIONS.—Baits used to attract flies in trap studies included human feces, cooked meats, milk-sugar mixture, and chopped fruits. Human feces attracted practically all species of flies that were attracted to the other baits and usually in much larger numbers. Table 1 summarizes data for the more prevalent species of flies taken in two traps baited with feces and operated 2 or more days each week from June 11 to August 17,[5] 1953. These data indicate the relative number of each species attracted to feces. One of these traps was located one-quarter mile from the Fort Churchill garbage dump, where uncontrolled fly breeding occurred, and the other was located at a test site 3 miles south of the post. The only known attractant already existing in the latter area was some bags of overwintered human feces. In addition to the 13 species listed in table 1, at least 13 others were identified from the miscellaneous other specimens attracted to traps baited with or hand collected on human feces or other baits.

In 1954 a trap set just outside the garrison mess and baited with feces was observed almost continuously from July 7 through August 2. The catch in this trap was markedly low as compared with those operated in areas off-post. About 87% of the total catch was of the two blowfly species *C. cadaverina* and *P. terrae-novae*, and the other 13% were miscellaneous muscids. No *Musca domestica* were taken. Similar results were obtained during three trap days in the same location in 1953. Results of the 1954 trapping are shown in table 2.

FLIES OBSERVED IN THE GARRISON MESS.—The garrison mess at Fort Churchill was inspected at frequent intervals during both seasons for the presence of adult

[5] Later seasonal collection records were made for several of these species and are noted in the discussion of individual species.

Table 1.—Summary of flies from two traps baited with human feces operated two or more days (total of 45 trap days) each week from June 11 to August 17, 1953.

SPECIES	TOTAL NO. TAKEN	INCLUSIVE DATES TAKEN	PERIOD OF GREATEST ABUNDANCE	LARGEST SINGLE DAYS CATCH—1 TRAP
Protophormia terrae-novae	6014	June 20–Aug. 17	July 5–Aug. 17	1244 on July 23
Cynomyopsis cadaverina	2420	June 11–Aug. 17	June 23–Aug. 17	134 on July 16
Hydrotaea bispinosa	1524	July 5–Aug. 17	July 14–Aug. 17	196 on July 23
Paregle radicum	1231	July 11–Aug. 17	July 14–Aug. 17	292 on July 23
Paregle mystacea	441	June 25–Aug. 17	July 14–July 24	100 on July 23
Fucellia ariciiformis	110	June 11–July 9	June 20–July 9	9 on June 24
Scopeuma furcatum, S. impudicum & *S. molle*	128	June 11–Aug. 17	Small numbers	10 on July 20
Muscina assimilis	47	June 17–Aug. 17	July 1–July 15	3 on July 9
Neoleria tibialis	35	July 15–Aug. 17	July 15–July 27	14 on July 16
Lucilia illustris & *Francilia alaskensis*	24	July 23–Aug. 17	Small numbers	8 on July 23
Sarcophaga sp.	16	July 14–Aug. 17	Small numbers	3 on July 16

Table 2.—Flies collected in one trap baited with human feces outside garrison mess at Fort Churchill—1954.

Period Covered	No. Trap Days	Average No. Trapped per Day			
		C. cadaverina	*P. terraenovae*	All Others	Total
July 7–13	6	2.0	0.3	0.5	2.8
July 13–20	7	7.3	2.4	2.1	11.8
July 20–August 2	6	6.3	7.0	1.0	14.3
Total	19	15.6	9.7	3.6	28.9

flies. Enough time to make a thorough inspection, approximately 20 to 30 minutes, was spent in checking it each time. The two Calliphorid species, *P. terrae-novae* and *C. cadaverina*, were more numerous in this mess in 1954 than in 1953. Whereas in 1953 most specimens of these species had been seen flying aimlessly around the rooms or trying to get out of windows, in 1954 they were commonly seen walking over meat in the storage room and butcher shop. *M. domestica* did not appear in the mess until August during both seasons. It was the prevalent species in the messhall after the outside temperature dropped appreciably. *M. domestica* exercised the typical house fly habit of crawling over food and was seen around indoor-garbage cans, sweets, and various other foods. A summary of the flies observed in this mess in 1954 is given in table 3. It is realized that daily variations in population were due in some measure to fly control activities of mess personnel; however these figures show the general trend of species prevalence.

Artificial Contamination of Flies with *Salmonella typhosa*.[6]—Three species of flies believed to be the most important potential carriers of enteric diseases in the Fort Churchill area, namely *Musca domestica*, *Cynomyopsis cadaverina* and *Protophormia terrae-novae*, were studied in the laboratory during August and September of 1954 in order to determine whether they remained contaminated with organisms of *Salmonella typhosa* following exposure. Specimens of each of the three species were caged and allowed access to a broth culture of *S. typhosa* for a period of 24 hours. Following this, the culture was removed and tests were made to determine the length of time the flies retained the organism. A petri dish containing cotton saturated with diluted condensed milk was placed in the cage for food. The flies were then macerated in selenite broth, which was then incubated for 24 hours. After incubation, the suspension was streaked on bismuth sulphite agar plates, incubated, and examined for *S. typhosa* colonies. Confirmation was made on triple sugar iron (TSI) agar slants. *S. typhosa* colonies were found to develop in the TSI agar slants from specimens of *M. domestica* up to 6 days following exposure and from specimens of *P. terrae-novae* up to 5 days following exposure. No recoveries were made from specimens of *C. cadaverina;* however this species was not studied as thoroughly as the others, due to difficulty of keeping it alive in captivity. Similar tests in 1953 did result in recovery of *S. typhosa* from *C. cadaverina* specimens 1 day following exposure.

Table 3.—Flies observed during weekly inspections in one garrison mess at Fort Churchill from May 20 to September 17, 1954.

Month	No. of Inspections	Number of Flies			
		C. cadaverina	*P. terrae-novae*	*M. domestica*	Total
May	1	0	0	0	0
June	4	33	8	0	41
July	3	29	3	0	32
Aug.	4	42	15	13	70
Sept.	3	0	0	48	48
Total	15	104	26	61	191

Information on Individual Species.—*Protophormia terrae-novae* (R.D.) (Family Calliphoridae) was the most abundant species of filth fly encountered. It was observed to breed extensively in animal carcasses, garbage and to a lesser extent in human feces. One fly was found active indoors as early as May 5, 1954. The earliest adult activity noted out-of-doors was on May 18, 1954, and the first oviposition was noted in the first week of June 1953. Some first brood larvae and pupae were caged and gave rise to adult emergence from July 27 to August 7, 1953. These adults laid eggs for a new brood, but it was not definitely determined whether the second brood developed to the adult stage during the same season. There is some indication that overwintering occurs in the adult stage as well as larvae, prepupae and pupae. One adult emerged on June 12, 1954 from a group of prepupae and pupae, part of which had overwintered in a block of frozen muskeg and part of which had overwintered in a bag of meat scraps. The period of greatest adult abundance was from about July 12 to August 20, with the peak about July 25. Millions of eggs were being laid on meat at the dump in late August. Adults were seen indoors in messes and kitchens only occasionally and were never encountered as a serious nuisance, either indoors or outdoors at food preparation areas. The latest record of adult activity was in late September. On August 10, 1953 examination of the rubbish at the garbage dump, where a bulldozer had stirred up the old garbage, revealed masses of pupae and larvae that had been under several inches of earth. In addition to these immature stages, mixed in with the dirt, rocks, cans, and crumpled paper were thousands of adult *P. terrae-novae*. It was not known whether these merely had not emerged to the surface of the ground or would normally remain in this habitat as adults throughout the winter.

Of 26 adults attracted to a trap baited with feces on June 29, 2 were male and 24 were female. Of 24 attracted to a meat-baited trap on July 9, 4 were male and 20 were female. While visiting at Coral Harbour on Southampton Island August 4, 1953, the senior author observed one adult specimen of what be believed was *P. terrae-novae*. Later Mr. Gooch of the Wild Life Department, Cornell University, presented the authors with two specimens of this species which he had reared from a goose carcass at the Boas river on Southhampton Island. The specimens had emerged August 18, 1953.

Cynomyopsis cadaverina (R.D.) (Calliphoridae) was the second most abundant species encountered. It was

[6] This phase of the study done through the cooperation of the bacteriologists assigned to the waste disposal study (see reference cited).

found further from its known breeding areas than *P. terrae-novae*. It was consistently taken in greater numbers than *P. terrae-novae* in meat-baited traps, and frequently it was more numerous in feces-baited traps located at the test site 3 miles or more from the post dump. It also was found breeding in animal carcasses and waste meat at the dump, as well as in human feces to a much lesser extent. Several adults were reared from pupae taken from feces at winter bivouac areas. Specimens were occasionally seen in btucher shops and other food preparation areas in the post messes, but usually they were flying aimlessly about or seeking escape through windows. It could not be considered a serious pest indoors, nor was it a nuisance at outdoor food preparation activities. It was active out-of-doors from the latter part of May until early October. Eggs laid early in June by the overwintering generation hatched in a few days, and the larvae matured and pupated by mid-July. The adults emerged from these pupae during the latter part of July and the first half of August. Eggs for a second generation were deposited in late July and August, but it was not possible to determine whether any of this group transformed to adults during the same season. There were indications of overwintering in the prepupal stage. Calliphorid prepupae were found in the soil at breeding areas in October 1953 and then again in May and early June 1954. Canadian entomologists reported finding hibernating adults of one or both of these Calliphorid species in piles of lumber under the snow in the Fort Churchill area in March.

A total of 300 specimens of *C. cadaverina* from a feces-baited trap on June 25, 1953, segregated by sex, revealed 18 male and 282 female flies. On July 10, 35 specimens from a trap baited with cooked meat revealed 2 males and 33 females. Of 37 flies reared from pupae from a sample of raw meat, 16 were male and 21 female.

About 50% of a large number of pupae of a mixed population of these two Calliphorid species from a dog carcass in July 1953 were parasitized and failed to emerge. On several occasions Calliphorid puparia were found to contain the pupa of an ichneumonid wasp parasite.

Hydrotaea bispinosa (Zett.) (Muscidae) was the third ranking species in abundance in 1953 and 1954 from traps baited with human feces, from traps baited with cooked meat, and from observations of fly populations at the post dump. It was first recorded from traps near the post dump about July 5 and continued fairly abundant throughout August. There was a distinct peak of adults in mid-July and apparently another in mid-August, which tends to indicate two generations of emerging adults during the season. No information was gained as to the probable overwintering stages. It was believed to be breeding at the post dump since large numbers of adults were observed there, and there was no other known breeding source in the area. This species was not reared from any of the several samples of larvae and pupae taken from the dump, or from a dog carcass or from human feces. It was not recorded from human habitations or in the vicinity of outdoor food preparation activities except for two specimens taken in a feces-baited trap just outside the garrison kitchen at Fort Churchill on July 24, 1953.

Paregle radicum (L.) and *P. mystacea* (Coq.) (Muscidae) were the fourth and fifth ranking species in abundance, respectively, in 1953 and 1954, as indicated by numbers taken in feces-baited traps and also from observations at the post dump during July. The former was first recorded from a trap on July 11, and the latter on June 25, 1953. The largest collection of both species was from a feces-baited trap near the post dump on July 23, when 292 and 100 specimens, respectively, were taken during the 24-hour period. These species continued fairly abundant through July then declined rapidly in August. During the peak period numerous specimens of both species were also taken in traps baited with cooked meat. Although they occurred in considerable numbers at the post dump, their small size and habits made them rather inconspicuous. They were thought to be breeding in the garbage at the dump. No information was obtained as to number of generations per season or overwintering stages. It is probable, since the first adult activity was so late in the year, that they pass the winter in an immature stage and produce one or not more than two broods in midsummer. They were not taken near human habitations or outdoor food preparation activities.

Fucellia ariciiformis (Holmg.) (Muscidae) was one of the first flies noted in early June 1953. It was taken on numerous occasions from feces- and meat-baited traps in June and early July. One specimen was collected at a window inside a lunch room at Fort Churchill on July 15. The largest number trapped was the first week in July; however they were never taken in large numbers, nor were they of apparent importance in these studies.

Scopeuma furcatum (Say), *S. impudicum* (Reiche), and *S. molle* (Beck.) (Scopeumatidae) were all taken from traps baited with feces and were otherwise observed only in close association with human feces in open areas. They were among the first species observed in early June 1953 and mid-May 1954 and continued to occur throughout the summer. They were rather difficult to collect by hand since they dart quickly from one fecal habitat to another when disturbed. They were seen in plastic bags containing overwintered human feces within the first day or two after the snow melted away from the bags, giving the impression that they had spent the winter under the snow as adults. However, larvae and pupae of an undetermined type of fly were observed in the bags at the same time and could have been the source of the newly emerged adults. They were noted to be an extremely wild species, and attempts to rear these aforementioned immature stages to adult or to get adults to oviposit in cages failed. The adults were never trapped in the immediate vicinity of human habitation nor were they observed in these areas. They were occasionally taken in traps baited with cooked meats when placed in the wild.

Muscina assimilis (Fall.) (Muscidae) was most prevalent in the vicinity of old latrine sites and at the post garbage dump throughout July and August 1953. At both of these habitats it was collected in moderate numbers in traps baited with human feces or cooked meat. The earliest trap record was June 17 and the latest was September 24. Adults were reared from numerous pupae taken from human feces and moist muskeg beneath the feces of the previous winter's bivouac latrine. Adults were also reared from pupae and larvae from garbage at the dump. This species was never evident in great numbers at either of these habitats and was never seen in the garrison

area or near other human habitations, or around human foods. In size and general appearance it somewhat resembles the common house fly. Of 63 specimens reared from human feces between July 7 and 15, 41 were male and 22 were female. Eleven specimens from a meat-baited trap at the post dump July 9 were all female.

Neoleria tibialis (Zett.) (Heleomyzidae) was taken in moderate numbers in traps baited with human feces, and to a lesser extent with cooked meat, during the latter half of July and throughout August 1953 and 1954. On June 24, 1953 several small dipterous larvae and numerous newly-formed bright red pupae were observed under a large cake of human feces in a screened building at the test site. This fecal mass had been there since the past summer and snow had recently melted leaving it uncovered. A screen cage was placed over the mass and about July 13 several hundred specimens of *N. tibialis* emerged. A carton of fresh feces was placed in the cage to induce oviposition, but as of August 7 no ova or new larvae were noted. In May 1954 larvae of *N. tibialis* were found in frozen muskeg at an old latrine site. They pupated in mid-June and began emerging the latter part of June. This species was not observed around human habitation or human foods.

Lucilia illustris Mg. and *Francilia alaskensis* Shan. (Calliphoridae) were both taken in traps baited with feces and also with cooked meat. Both were taken in the Fort Churchill area as well as at Limestone Camp some 150 miles south of Fort Churchill. The two species are listed together here since their similarity in appearance made it impractical to separate them during the conduct of these studies. One or both species were collected throughout the period July 17 to August 18, 1953 but in very limited numbers.

Musca domestica L. (Muscidae) was thought by Canadian entomologists, working in the Fort Churchill area on other problems, to be nonexistent in that area. To the best of their knowledge, it had never been reported there. One specimen, however, was taken along with 619 other fly specimens July 15, 1953 from a trap baited with human feces, located at a winter bivouac latrine site 5 miles south of Fort Churchill. This is the only occasion on which it was taken in a trap with the exception of the immediate vicinity of the post garbage disposal area. On July 21, one specimen was hand collected inside a small wooden privy over a dug latrine at Bird Cove, some 5 miles southeast of Fort Churchill. On July 23, five specimens were caught in the garrison mess at Fort Churchill. On August 3 numerous adults were seen at the post garbage dump. It gradually increased in abundance at the dump throughout August and was undoubtedly breeding there, although no immature stages were ever definitely identified. This species never accounted for more than 10 to 20% of the entire fly population at the dump in 1953. The earliest observation of the house fly at this dump in 1954 was on June 15, but it did not increase in population appreciably until late in August, when it became the dominant species, comprising an estimated 50 to 70% of the total fly population. The adults continued to be prevalent at the dump in 1954 until the middle of September, then declined rapidly. During both seasons, *M. domestica* began to show up in the garrison messes in August and continued to increase in numbers into late September and was taken in one mess as late as October 8. By temperate zone standards, this species was not considered a serious pest in the messes at Fort Churchill; however it became distinctly more abundant in 1954 than it was in 1953, and conceivably could be becoming more satisfactorily adapted to existence in that area. It was not positively determined that this species overwinters in the area, but specimens found as early as mid-June indicate that it does. A few specimens were noted in a small restaurant in the town of Churchill. Of 27 specimens taken with an insect net at the dump in early August, 16 were males and 11 were females. Four of five specimens from a messhall collection were females.

This species at present does not present a problem under field conditions as a breeder in human feces or as a pest at bivouac or camping activities. The entire population of the species appears to be associated with garbage disposal at Fort Churchill, and possibly very limited breeding occurs in the small town of Churchill. The Fort Churchill garrison has a water-borne sewage system except for a few septic tanks in the older portion of the post. The waste from the latter was, at least once during the summer 1953, pumped out and emptied on the surface of the ground at the dump, thus adding to the fly problem. The water-borne sewage flows into the Hudson Bay and is disposed of through wave and tidal action.

Copromyza annulus (Walk.) (Sphaeroceridae) is a small fly which was seen frequently on human feces at the old winter bivouac latrine sites and also on stale bread and other foods left in these bivouac areas. They were noted at the time of the earliest field observation the first week in June 1953, being present on the overwintered feces as soon as the snow melted away. They were observed throughout the summer. It was not recorded near human habitation or food preparation areas.

Graphomya maculata (Scop.) (Muscidae) was taken on numerous occasions at the test site near Fort Churchill in July and August 1953 in traps baited with feces. It was never noted in other situations.

Sarcophaga lherminieri (R.D.) (Sarcophagidae). Several specimens were taken at Limestone Camp 150 miles south of Fort Churchill in a trap baited with feces on July 18, 1953. No definite record was made as to its presence at Fort Churchill. It was not observed at food preparation activities.

Helina lucorum (Fall.) (Muscidae) was taken at a point 5 miles south of Fort Churchill on July 15, 1953 in a trap baited with feces.

Phaonia errans (Mg.) (Muscidae) was taken July 18, 1953 at Limestone Camp 150 miles south of Fort Churchill, in a trap baited with feces.

Myospila meditabunda (F.) (Muscidae) was taken on July 15 and 17, 1953 at the test site 5 miles south of Fort Churchill, and on July 18, 1953 at Limestone Camp 150 miles south of Fort Churchill. All specimens were from a feces-baited trap.

Piophila vulgaris Fall. and *P. affinis* Mg. (Piophilidae) were both hand collected August 7, 1953 near Fort Churchill, from a bag containing banana and apple. Several specimens of *P. vulgaris* were also hand collected in the same area on July 13, from a bag containing overwintered feces.

Thaumatomyia trifasciata (Zett.) (Chloropidae) was

taken July 10, 1953 at a winter bivouac latrine site 5 miles south of Fort Churchill, from a feces-baited trap. Several specimens were taken.

Scoliocentra fraterna (Iw.) (Heleomyzidae) adults emerged in considerable numbers on August 3, 1953 from the carcass of a dog which was also heavily infested with larvae of *C. cadaverina* and *P. terrae-novae*.

Scopeuma lanatum (Lundb.) (Scopeumatidae). One specimen was hand collected August 4, 1953 at a dump at which human feces was being disposed of near Coral Harbour on Southampton Island in the northern end of the Hudson Bay.

Dolichopus sp. Several specimens were taken July 28, 1953, near Fort Churchill, from a feces-baited trap.

Symphoromyia sp. One specimen was taken July 18, 1953 at Limestone Camp 150 miles south of Fort Churchill, from a feces-baited trap.

SUMMARY.—Twenty-seven species of filth flies are recorded from studies conducted in the vicinity of Fort Churchill, Manitoba, Canada in 1953 and 1954. Studies on biology, habits, abundance, seasonal occurrence and probable importance in human enteric disease transmission are reported for several species. *Musca domestica* and two species of Calliphorid flies *C. cadaverina* and *P. terrae-novae* were observed frequenting indoor messes in limited numbers. None were observed in any significant numbers at food preparation or food serving activities at troop bivouacs or outdoor camps. Human feces left on the surface of the ground at winter bivouac snow latrines, and also fresh feces, appeared to be a negligible source of filth flies of probable medical importance during the conduct of these studies.

REFERENCE CITED

Final Report, Sub-Task of Project 6-61-13-004, Human Wastes in Arctic and Sub-Arctic February 1952–September 1954, of the Army Environmental Health Laboratory of the Army Medical Service, Army Chemical Center, Maryland.

Greenbug Damage to Winter Wheat as Affected by Preceding Crop[1]

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The greenbug, *Toxoptera graminum* (Rond.), has been a major pest of wheat in the Central and Southeastern States for many years. It causes some damage every year and several severe outbreaks have occurred. Since 1942, two serious outbreaks have occurred in the High Plains of Texas. Various workers have indicated that wheat following legumes may be somewhat more tolerant to greenbugs than wheat following other crops. Therefore, it seemed advisable to test the fecundity of this pest in relation to tolerence of wheat plants grown in soil from wheat and alfalfa fields under greenhouse conditions.

Painter (1951) cites a number of papers on the relation of soil fertility to insects. He states that "each species of insect and often each host-plant species or variety constitutes a separate problem." There is little specific work on the effects of plant nutrients or soil fertility to the greenbug. Haseman (1946) indicated that the greenbug has a high nitrogen requirement and will not reproduce as well on small grains grown on land deficient in nitrogen and iron. However, investigations by Arant & Jones (1951) showed greenbug populations to vary inversely with the amount of nitrogen applied to oats. Blickenstaff *et al.* (1954) also found that in oats and rye the population of greenbugs decreased as the rate of nitrogen was increased. In the present work, the effort has been to carry on a study relating to soil condition resulting from crop rotation.

MATERIALS AND METHODS.—Pullman silty clay loam was obtained at 1-foot intervals to a depth of 4 feet from land growing wheat and alfalfa. The legume land, which had been in alfalfa for 5 years previous to 1954, was approximately 1 mile from the wheat land. Each 1-foot interval of the two soils, an equal parts mixture of the first and fourth foot interval, and an equal parts mixture of all soil intervals were placed in 8-inch pots. The pots were arranged in a split plot design and replicated four times. The test was conducted in 1954 and again in 1955. Pawnee wheat was planted and later thinned to four plants per pot for the 1954 test and to 10 plants per pot for the 1955 test. Whole plots consisted of four pots from one soil interval. Each whole plot was divided into subplots consisting of two pots of either alfalfa or wheat soil. One pot of each subplot was infested with greenbugs and the other left as a noninfested check.

One-half gallon mason jars, with the bottoms removed, were used as plant cages. The jars were capped with 70-mesh brass screen. Noninfested plants as well as infested were caged to provide similar growing conditions. The wheat plants were each infested, when about 3 inches tall, with one adult greenbug.

Population counts were taken 30 days after infestation. Counts were made by clipping, at random, half the plants of each infested pot and brushing the greenbugs from the plants onto a white cardboard where they were counted. Green weights of the infested and noninfested plants were taken for comparison at the time of count.

The soils were analyzed for organic matter, available phosphorus, potash and lime. Total nitrogen content of the soils was calculated from the organic matter data using a C:N ratio of 10:1.

RESULTS AND DISCUSSION.—The mean number of greenbugs on the wheat plants grown in the various soil intervals in tests conducted in 1954 and 1955 are presented in table 1. The means squares from the analysis of vari-

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Table 1.—Mean number of greenbugs on wheat plants grown in soil taken at different depths from wheat land and alfalfa land.

Soil Interval or Depth in Feet	Mean Number of Greenbugs					
	1954[a]			1955[b]		
	Wheat Land	Alfalfa Land	Mean	Wheat Land	Alfalfa Land	Mean
1	135	215	175	625	876	751
2	142	120	131	298	247	273
3	106	164	135	238	376	307
4	110	110	100	281	217	249
1+4	256	281	269	406	458	432
1+2+3+4	196	181	189	593	779	686
Mean	158	179		407	492	

[a] Number of greenbugs on two plants.
[b] Number of greenbugs on five plants.

ance, table 2, indicate that greenbug populations on wheat of different soil levels differed significantly. The populations were greater on plants grown in the first foot of soil and the two soil mixtures. The mean number of greenbugs on the plants grown in soil from alfalfa land was significantly greater than on the plants grown in wheat land soil.

Infested plants of all soil intervals were severely damaged by the greenbugs (Table 3). Both the infested and noninfested plants grown in soil from the first foot interval and the two mixtures had higher mean weights than the plants grown in soil from the second-, third- or fourth-foot intervals. Plants grown in soil from alfalfa land were significantly heavier than plants grown in wheat land soil. Although infested plants grown in soil of the first-foot interval and the two mixtures were heavier than those grown in the second-, third- and fourth-foot intervals, they were in general lower in weight in comparison with the appropriate check plants. This difference was significant as shown by the interactions (I×S), table 4. Infested plants grown in alfalfa soil, in comparison with the appropriate check, were only slightly lower in weight than infested plants grown in wheat soil. The greater number of greenbugs produced on the more vigorous plants, table 1, probably accounts for the larger comparative reduction in weight of the more vigorous plants.

Table 2.—Analysis of variance of the number of greenbugs on infested plants in tests conducted in 1954 and 1955.

Due to	d.f.	Mean Squares	
		1954	1955
Replications (R)	3	36,811	81,228
Soil Intervals (S)	5	26,413**	381,898**
R×S (Error a)	15	4,408	26,083
Crop Land (C)	1	5,376	87,722*
C×S	5	3,408	32,998
Error b	18	6,235	11,531
Total	47		

Table 4.—Analysis of variance of green plant weight in tests conducted in 1954 and 1955.

Due to	d.f.	Mean Squares	
		1954	1955
Replications (R)	3	0.1507	0.1717
Soil Intervals (S)	5	0.6557**	3.2840**
Error a	15	0.0711	0.1942
Crop Land (C)	1	0.2817*	1.7146**
C×S	5	0.0544	0.4095
Error b	18	0.0345	0.1664
Infestations (I)	1	6.0702**	26.8711**
I×S	5	0.2325**	0.5157**
I×C	1	0.0011	0.2252
I×C×S	5	0.0500	0.0980
Error c	36	0.0298	0.1449
Total	95		

Soil analyses with respect to total nitrogen and available phosphorus content are presented in table 5. The percentage of nitrogen was greater at the first-foot interval. The nitrogen was also generally higher in the alfalfa soil in comparison to the wheat soil. There was more available phosphorus in the deeper soil intervals and also a slightly higher phosphorus content in the alfalfa soil than in the wheat soil.

The relationships existing between greenbug populations and noninfested plant weights, soil nitrogen content and available phosphorus are shown by correlation and regression coefficients in table 6. It is apparent that the

Table 3.—Mean green weights in grams of infested and noninfested plants grown in soil taken at different depths from wheat land and alfalfa land.

Soil Interval or Depth in Feet	1954[a]						1955[b]					
	Wheat Land		Alfalfa Land		Mean		Wheat Land		Alfalfa Land		Mean	
	Inf.	Check	Inf.	Check	Inf.	Check	Inf.	Check	Inf.	Check	Inf.	Check
1	0.55	1.25	0.54	1.37	0.55	1.31	0.73	1.75	1.42	2.75	1.08	2.25
2	.27	.64	.38	.72	.33	.68	.63	1.24	.59	1.89	.61	1.57
3	.29	.68	.48	.75	.39	.72	.29	.93	.58	1.28	.44	1.11
4	.24	.66	.48	.63	.36	.65	.42	1.22	.45	1.20	.44	1.21
1+4	.34	1.18	.64	1.50	.49	1.34	.96	2.45	.99	2.85	.98	2.65
1+2+3+4	.68	.92	.44	1.05	.56	.99	1.02	2.19	1.03	2.03	1.03	2.11
Mean	.40	.89	.49	1.00	.45	.95	.68	1.63	.84	2.00	.76	1.82

[a] Weights of two plants.
[b] Weights of five plants.

Table 5.—Mean total nitrogen content and available phosphorus of the wheat and alfalfa soils used in the 1954 and 1955 tests.[a]

Soil Interval or Depth in Feet	Per Cent Nitrogen		P_2O_5 (p.p.m.)	
	Wheat	Alfalfa	Wheat	Alfalfa
1	0.0606	0.0911	2	4
2	.0377	.0360	15	13
3	.0220	.0319	25	43
4	.0214	.0267	27	53
1+4	.0405	.0599	14	28
1+2+3+4	.0355	.0464	19	30

[a] Potash and lime content in these soils were above 240 and 2800 p.p.m. respectively; differences that may have existed among soil intervals above these levels were not determined.

Table 6.—Regression and correlation coefficients of greenbug populations on noninfested plant weights, per cent nitrogen and available phosphorus in soil and of noninfested plant weight on per cent nitrogen and available phosphorus in soil.[a]

	Green Plant Weight	Number of Greenbugs on Plants
Green plant weight, in grams		217.35**
		.799**
Per cent nitrogen in soil	18.68**	5288.60**
	.800**	.823**
P_2O_5 in soil (p.p.m.)	−.0141	−3.63
	−.450	−.425

[a] Regression coefficients upper figure.

number of greenbugs was positively associated with plant vigor and percentage nitrogen in the soil. This is in agreement with the data presented by Haseman (1946). Plant vigor in this test was related to the amount of nitrogen in the soil. The negative coefficients obtained in the case of available phosphorus are probably the results of a lack of nitrogen rather than to a plentiful supply of available phosphorus.

Summary and Conclusions.—Studies were conducted on the effects of soil taken from both wheat and alfalfa land on the populations of *Toxoptera graminum* and its damage to wheat. Soil was taken at 1-foot intervals to the depth of 4 feet. Each soil interval, an equal parts mixture of all soil intervals and an equal parts mixture of the first- and fourth-foot intervals were placed in 8-inch pots. Pawnee wheat grown in the different soil intervals was infested with greenbugs. Population counts and green plant weights were later taken. The pots were arranged in a split plot design and replicated four times. The soils used were analyzed for organic matter, available phosphorus, potash and lime.

The results indicated that increased greenbug populations were positively associated with high nitrogen levels in the soil and increased plant vigor under conditions of this experiment. Wheat grown in alfalfa soil was found to be more tolerant to greenbugs, inasmuch as it supported greater populations, than wheat grown in soil taken from wheat land. The first-foot intervals and the mixtures of both wheat and alfalfa soil supported more greenbugs than the second-, third- or fourth-foot intervals.

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Control of Aphids on Cigar-wrapper and Flue-cured Tobacco[1]

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The widespread damage to tobacco by the green peach aphid (*Myzus persicae* (Sulz.)) in 1947 precipitated an intensive testing program for effective insecticidal control of this pest. After the discovery of parathion as an efficient aphicide, interest in other materials subsided. Recently, however, other insecticides, some of which are considerably safer to man than parathion, have been tested against the green peach aphid on tobacco. These tests are of added interest because of the possibility of the existence of strains of aphids resistant to parathion (Anthon 1955).

In the cigar-wrapper district of Florida, where tobacco fields (shades) are grown under cloth tents, it is the usual procedure to apply insecticides by airplanes after the plants are about one-half grown. Although growers have been well satisfied with the control of both budworms and aphids from airplane applications through shade cloth, no studies have been made to determine the amount of insecticide retained by the shade cloth top.

The effectiveness of certain newer materials on aphids attacking cigar-wrapper and flue-cured tobacco is herein reported. In addition, the screening effect of shade cloth to airplane applications of insecticides is discussed.

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REVIEW OF LITERATURE.—Creighton & Gresham (1947) first reported control of green peach aphids on tobacco with parathion in the cigar-wrapper district of Florida. This work was confirmed by Wilson *et al.* (1948) who also pointed out that lindane and rotenone gave satisfactory control of aphids in plant beds. Chamberlin (1949) obtained good control with parathion alone and in combination with DDT but did not obtain adequate control of heavy infestations of aphids with lindane on cigar tobacco in Florida. None of the workers in Florida obtained satisfactory results with DDT or TDE. Turner (1949) and Woodruff (1951) reported tests in the cigar-wrapper district in Connecticut where excellent control was obtained with parathion. They also indicated that the use of DDT reduced aphid infestation to a marked extent. Kring (1955) reported that malathion gave satisfactory control in the Connecticut district against a light infestation of aphids.

On flue-cured tobacco, Dominick (1949) reported adequate control of aphids with both parathion and BHC. Kulash (1949) found that parathion was the most promising aphicide tested on flue-cured tobacco. He also indicated that aphids are not a problem in flue-cured tobacco during most years.

EXPERIMENTAL PROCEDURE.—Tests on shade-grown tobacco were conducted in 1953 and 1954 in the cigar-wrapper district of Florida. In 1953 the tests were conducted on plots 82 feet long and four rows wide, and in 1954 the plots were 64 feet long and three rows wide. Each treatment was replicated three times in a randomized block design. Applications were made with rotary hand dusters on a weekly schedule as recommended in that district. The rate of dust application was gradually increased from eight to 25 pounds per acre as the plants increased in size. In 1953, each plot was artificially infested a few days after transplanting by placing aphid-infested leaf sections containing 10 to 30 aphids on each plant in the two center rows of each plot. In addition, two 6-foot square pieces of shade cloth were removed from each wall of the shade to provide windows for natural infestation. Thus, re-infestation could occur either through the windows cut in the wall of the shade or from plots on which aphids were not controlled. The 1954 experimental plots were infested in the same manner as in 1953 except that each plot was artificially infested two additional times during the season at about 3-week intervals.

In the Florida tests, comparative counts were made by determining the number of aphids on 10 plants in each plot at weekly intervals throughout the season. Counts were usually made 3 days after treatment. During the early part of the season the number of aphids on the entire plant was determined. As the season progressed, the aphids on bud leaves of pre-determined sizes were counted. Therefore the counts obtained were comparative rather than absolute.

Tobacco from these plots was harvested and graded according to commercial standards. In the shade district of Florida, leaves which are badly infested by aphids or which have numerous holes from foliage-consuming pests are not deemed suitable for harvest. Therefore, the decrease in yield shown by certain treatments represents both aphid and budworm injury. Results of tests conducted on shade-grown tobacco in 1953 and 1954 are shown in tables 1 and 2, respectively.

In 1954, experiments on flue-cured tobacco were conducted against natural aphid infestations. In one experiment (Table 3), there were three replications of each treatment, and each plot was six rows wide and 83 feet long with three guard (untreated) rows between plots. Dusts were applied with a rotary hand duster. Sprays were applied with a self-propelled, high-clearance sprayer operated at 60 pounds pressure. Five hollow cone nozzles were used per row, and the spray was delivered at the rate of 27 gallons per acre. The tobacco plants in these plots were 3 to 4 feet tall. Aphids were counted at the indicated intervals from a sample consisting of one leaf, chosen at random, from the upper half of each of five plants in each plot.

In another 1954 experiment (Table 4), spray and dust treatments of both parathion and malathion were evaluated in two separate tests. In the spray tests, plots consisted of one row of tobacco 78 feet long. There were four

Table 1.—Comparative effectiveness of weekly applications of insecticide dusts applied against the green peach aphid attacking shade-grown tobacco. Quincy, Florida, 1953.

Material	Average No. Pounds Dust per Acre per Application	Number[a] of Aphids on Dates Shown							Yield Pounds per Acre	Grade[c] Index
		4/13[b]	4/17	4/24	5/1	5/8	5/15	5/22		
1% parathion	12	415	26	0	0	0	0	0	937	0.257
1% parathion+10% TDE[d]	14	623	108	0	6	1	0	0	1,000	.350
2.5% Metacide[e]+10% TDE	10	586	0	0	7	11	0	0	998	.306
5% malathion	13	1,026	4	0	2	24	0	0	1,032	.263
2.5% malathion+10% TDE	12	671	259	0	9	269	192	161	976	.263
1.5% lindane+10% TDE	12	632	145	243	244	1,192	1,147	684	825	.271
Check	—	650	1,303	3,439	5,924	7,900	112[f]	26	739	.217
L.S.D. at 5% level									82	.106
L.S.D. at 1% level									111	.143

[a] Total on three replicates.
[b] Pre-treatment count.
[c] A high grade index indicates high quality tobacco.
[d] Standard treatment.
[e] Metacide is a mixture whose active ingredient contains 80% methyl parathion+20% parathion.
[f] Treated with 1% parathion dust on 5/11.

Table 2.—Comparative effectiveness of weekly applications of insecticide dusts applied against the green peach aphid attacking shade-grown tobacco. Quincy, Florida, 1954.

Material	Average Pounds per Acre per Application	Number[a] of Aphids on Dates Shown							Yield Pounds per Acre	Grade[c] Index
		4/6[b]	4/13	4/21	4/28	5/5	5/12	5/20		
1% parathion+10% DDT[d]	14.8	296	0	0	0	0	0	15	1,442	0.646
1% parathion+10% DDT (biweekly)	14.3	225	0	0	475	23	0	5	1,441	.708
2.5% Chlorthion+1% endrin	19.5	248	0	0	0	0	0	0	1,395	.619
5% malathion+1% endrin	14.5	249	0	0	0	0	0	8	1,385	.655
1% endrin	16.2	297	180	385	319	229	905	1,285	1,258	.679
2% endrin	17.6	228	2	0	96	46	397	326	1,442	.641
10% Strobane	19.5	254	1,347	331	959	1,451	7,570	9,250	804	.237
5% Diazinon+2.5% isodrin[e]	13.4	295	0	0	0	0	0	0	1,440	.639
Check	—	289	3,220	9,900	12,550	22,750	33,800[f]	11,060	346	.340
L.S.D. at 5% level									209	.155
L.S.D. at 1% level									282	.209

[a] Total on three replicates.
[b] Pre-treatment counts.
[c] A high grade index indicates high quality tobacco.
[d] Standard treatment applied weekly.
[e] F. S. Chamberlin, Entomology Research Branch, U. S. Dept. Agric. (1953 unpublished data) showed that 2% isodrin dust had no appreciable effect upon the green peach aphid on shade-grown tobacco.
[f] Dusted with 6 lbs of 1% parathion dust per acre immediately after aphid counts.

Table 3.—Comparative effectiveness of TDE and endrin dusts and sprays against green peach aphid attacking flue-cured tobacco. North Carolina, 1954.

Material	Formulation	Pounds Actual per Acre	No. Aphids Before Treatment[a]	Per Cent Reduction of Aphids[b] After (Days)		
				2	3	7
TDE	25% E.C.	0.7	2,608	41	36	28
	10% dust	3.7	1,585	40	43	62
Endrin	19.5% E.C.	.14	1,479	42	41	41
	19.5% E.C.	.29	2,607	80	80	82
	1.5% dust	.23	1,632	60	61	79
Check		—	1,564	21	5	5

[a] Total number of aphids on 1 marked leaf of 5 plants in each plot.
[b] Average of three replicates.

replications of each treatment with two guard rows separating plots. The plants were 2 to 3 feet in height at time of treatment. The spray treatments were applied with a hand-operated, compressed air sprayer. Ten plants in each plot were marked at random, and the total number of aphids on each plant was counted at the desired intervals. The dust plots were two rows wide and 78 feet long. There were three replications of each treatment and three guard rows between plots. The plants were 3 to 4 feet in height at time of treatment. The dusts were applied at 30 pounds per acre with a rotary hand duster. Five random plants were chosen in each plot, and the aphids on one marked leaf on each plant were counted at the desired intervals.

In 1955, the plots were infested 1 week after transplanting by placing a leaf section containing about 25 aphids in the bud of each tobacco plant. The plots were two rows wide and 60 feet long. There were three replications with two guard rows between plots. The materials were applied with a high clearance sprayer operated at 60 pounds pressure. Seven hollow cone nozzles were used per row, and the spray delivered at the rate of 35 gallons per acre. The plants in these plots were not uniform in size ranging from about 4 feet to nearly 7 feet tall. Ten plants in each plot were marked and the aphid infestation determined on the first five terminal leaves which were at least 2 inches long. The results of these tests are shown in table 5.

The tests to determine the effectiveness of airplane dusting and spraying through shade cloth in the cigar-

Table 4.—Comparative effectiveness of malathion and parathion sprays and dusts against green peach aphid attacking flue-cured tobacco. North Carolina, 1954.

Formulation	Pounds Actual per Acre	No. Aphids Before Treatment[b]	Per Cent Reduction of Aphids[a] After (Days)				
			1	2	3	7	14
SPRAYS W.P.							
15% parathion	0.15	2,653	45	—	97	98	91
25% malathion	0.75	4,789	54	—	97	97	98
Check		3,541	−9[c]	—	20	−45[c]	−63[c]
DUSTS							
1% parathion	0.30	1,966[d]	—	96	99	99	
4% malathion	1.20	3,018[d]	—	98	99	98	
Check	—	3,449[d]	—	43	44	45	

[a] Average of three replicates.
[b] Total number of aphids on 10 plants in each plot.
[c] Represents an increase of aphids over the pre-treatment counts.
[d] Total number of aphids on 1 marked leaf of each of 5 plants in each plot.

Table 5.—Comparative effectiveness of insecticidal sprays applied against aphids attacking flue-cured tobacco. North Carolina, 1955.

Material	Pounds Actual per Acre	No. Aphids Before Treatment[a]	Per Cent Reduction of Aphids (Average of 3 Replications)	
			48 Hrs.	7 Days
Endrin	0.4	24,121	72	88
Parathion	0.25	27,785	94	98
Malathion	1.0	21,875	94	97
Chlorthion	1.0	22,545	98	99
Check	—	12,380	−10[b]	53

[a] Total number of aphids on upper 5 leaves of 10 plants in each plot.
[b] Represents an increase in aphids over pre-treatment count.

Table 6.—The effect of shade cloth as a screen to airplane applications of DDT to tobacco. Quincy, Florida, 1954.

TYPE OF CLOTH COVER	FORMULATION	CONDITION OF SHADE-TOP	PER CENT OF DDT RETAINED BY CLOTH TOP[a]
Double Conn.	10% dust	dry	34
Florida heavy	10% dust	dry	38
Double Conn.	10% dust	damp from dew	66
Florida heavy	10% dust	damp from dew	61
Florida heavy	25% E.C.	dry	59
Florida heavy	25% E.C.	dry	48
			S.E.±13

[a] Average of three replications.

wrapper area (Table 6) were conducted by catching the insecticides immediately above and below the shade cloth top and analyzing the residues chemically. Sprays were applied at about 1.5 pounds of actual DDT, and dusts were applied at about 30 pounds of 10% DDT dust per acre. Immediately before dusting or spraying, dampened pieces of filter paper (0.394 sq. ft.) were fitted to 9-inch diameter pie pans located at three equidistant points in the southeastern corner of each field. Four pans were fastened to the top of the shade cloth in each location, and four other pans were fastened a foot below the top. The pans were equally spaced 1 foot from the center of each location, and the pans placed below the cloth were always located within 8 feet of the pans placed above the cloth. After treatment, the filter papers above and below the cloth were placed in ½-gallon jars and brought into the laboratory where 450 ml. of benzene were added to each jar. The jars were stored at 40° F. until chemical analyses could be made. The residues were analyzed by the modified Schechter-Haller method.

There were two general types of shade cloth used in these experiments. The Connecticut shade cloth is woven with eight threads per inch in one direction and 10 in the other, and 1 pound contains 11 square yards. The shade is usually doubled when it is put on the shade top and is called Double Connecticut. The other type is the Florida heavy cloth which is not doubled and is woven with 16 threads per inch in each direction. One pound of the Florida heavy cloth contains 7 square yards.

RESULTS AND DISCUSSION.—According to data from the 1953 test, as shown in table 1, 2.5% metacide and 5% malathion offer possibilities as substitutes for parathion in controlling aphids. Although 2.5% malathion dust did not give outstanding control of aphids, the 5% malathion dust gave as good control as the standard treatment. In agreement with Chamberlin (1949), lindane does not offer enough protection against aphids on cigar-wrapper tobacco in Florida to be of commercial value. Untreated tobacco suffered such a heavy loss from aphid attack that one parathion treatment was applied to the check plots on May 11 in order to save some tobacco for subsequent smoking tests. Figure 1 shows the stunting effect of heavy aphid infestations on shade tobacco about 3 weeks after planting.

Before examining the 1954 data on cigar tobacco, attention is called to the extremely severe aphid attack under which the materials were tested, owing to use of multiple, artificial infestations. Table 2 shows that Chlorthion-endrin, malathion-endrin, and Diazinon-isodrin dust mix-

FIG. 1.—Young cigar-wrapper tobacco stunted by aphids. Left untreated, right treated.

tures give about the same protection against aphids as parathion-TDE when used at the rates shown. On the basis of subsequent experience with these materials, it is believed that the phosphates were mainly responsible for reducing the aphid population. These data also show that applications of parathion every 2 weeks prevent aphids from building up to injurious infestations in the cigar-wrapper district of Florida. Two per cent endrin dust offered considerable protection to the plants until late in the season; 1% endrin, although never preventing some aphids from becoming established, kept down the infestation to a marked degree. Ten per cent Strobane dust was unsatisfactory for aphid control. The decrease in yield and grade in the Strobane-treated plots can be attributed primarily to aphid damage because this material controlled budworms satisfactorily.

In regard to the experiments conducted on flue-cured tobacco in 1954, data in table 3 show that TDE, used as either a spray or a dust, has some effect on aphids but does not approach commercial control. Endrin reduced aphid populations appreciably at a dosage of 0.29 lb. per acre but was much less effective at lower dosages. Table 4 shows that malathion offers good promise as a substitute for parathion in the flue-cured tobacco district.

In 1955 (Table 5) malathion again gave very good control, and Chlorthion was also shown to be an excellent aphicide. As in 1954, endrin offered appreciable protection from aphids. Hence, it appears that growers might take advantage of the partial protection against aphids offered by endrin when selecting a material for use against other insects. In 1953 and 1954 all the materials tested were very toxic to lady beetles and syrphids, important predators of aphids in the tobacco section.

Shade cloth had considerable screening effect on both dust and spray treatments applied by airplane. Data presented in table 6 show that about one-third of applied dust material is retained by a dry shade cloth and two-thirds by a damp shade cloth. About one-half of airplane-sprayed material is retained by a dry shade cloth. The type of shade cloth had little effect on the penetration of airplane applications. In these experiments the insecticide was measured at very short intervals after treatment, and it is possible that the insecticide caught by the cloth could subsequently drop through to the tobacco foliage.

SUMMARY.—Tests conducted on shade-grown tobacco in Florida showed that 1% parathion, 2.5% Metacide, 5% malathion, 2.5% Chlorthion, and 5% Diazinon dusts gave excellent control of aphids. Endrin dust offered appreciable control but was not considered to be satisfactory. Lindane and Strobane were of little use in preventing heavy aphid infestations.

Tests conducted on flue-cured tobacco showed that parathion, malathion, and Chlorthion gave excellent control of heavy aphid infestations. Although less effective than the above materials, endrin reduced aphid populations considerably.

Airplane applications of dusts and sprays in the shade-grown tobacco district of Florida were screened to a marked extent by the shade cloth tops. Dew-laden shades retained more dust from airplane applications than dry shades. Dusts were more easily passed by the cloth than sprays when shades were dry.

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THOMAS SAY FOUNDATION, VOLUME II

A supply of Volume II, Plecoptera or Stoneflies of North America, by J. G. Needham and P. W. Claassen has recently been located. All five volumes that have been published by the Foundation are now available. Copies of Volume II sell for $5.00 postpaid in North America and U. S. Possessions, and for $5.50 elsewhere.

On the Biology and Control of *Drosophila* on Tomatoes for Processing[1]

WILLIAM E. COLLINS,[2] *New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick*

On certain occasions, *Drosophila* spp., often called sourflies, pomace flies, or vinegar flies, become so abundant that they pose a serious economic problem to the canning industry. Since the presence of eggs and maggots in the canned product is prohibited, packs of tomatoes are subject to seizure and destruction. In recent years, the tomato industry has been plagued by this pest which in the later part of the season becomes very abundant in the field and at the canning factory. Control of this pest is complicated by such factors as (1) enormous populations, both in the field and at the processing plant, (2) numerous sources of reinfestation, and (3) the limitation of insecticides which may be used because of residue hazards. With this in mind, the observations were made to obtain information on the species involved, seasonal abundance, relation of fruit maturity to egg deposition and control procedures. Although the information obtained is incomplete, some insight into the nature of this insect pest and its possible control was obtained.

SPECIES AND ABUNDANCE IN THE FIELD.—Efforts have been made by several workers to develop techniques for the estimation of fly abundance in the field. Ditman *et al.* (1936) used tomatoes which had been cut in half and placed in the field. After a 24-hour exposure period, the average number of eggs per square inch of exposed flesh was recorded. Dobzhansky (1943) baited the bottom of drinking cups with a layer of fermenting banana and at suitable time intervals removed the flies from the cups and counted them. Spencer (1950) has shown, however, a disadvantage in the use of baits in that one species is attracted more than another, and thus the picture of species abundance is distorted. Michelbacher *et al.* (1952) used ripe tomatoes in which a 1½-inch slit had been made on one side. After a 24-hour exposure period, the section of the fruit along the slit was removed and examined. The egg deposition on the exposed or trap tomatoes was found to be proportional to the fly abundance. Since egg deposition was of primary concern in our studies, this method was thought best for our purpose.

During the 1952 season, observations were made in a field being used for fungicidal experiments. Test fruits cut in a cross-shaped pattern were put in strawberry boxes and placed under the foliage of the tomato plants. The tomatoes were examined under a binocular microscope to determine the egg deposition. Twenty-four-hour egg deposition records were made from September 8 until September 23. By the latter date the population had reached such tremendous proportions accurate counting of the eggs became almost impossible. On September 20, however, it was estimated that 1600 eggs were deposited per fruit.

During the 1953 season, observations were carried out in a field that had not been treated with insecticides or fungicides. This situation offered an opportunity to observe the natural build-up of the various species. The procedure for estimating egg deposition was the same as in the preceding year. Four species were found in abundance, namely, *Drosophila tripunctata*, Loew; *D. melanogaster*, Meigan; *D. simulans*, Sturtevant; and *Scaptomyza adusta* Loew. *Drosophila tripunctata* was relatively abundant during the middle part of August, but disappeared almost completely by the end of the month and ceased to be of concern from the standpoint of fruit contamination. The seasonal abundance of *Scaptomyza adusta* closely paralleled that of *D. tripunctata*.

The eggs of *Drosophila melanogaster* were collected first on August 19. From August 23 until almost the end of October this was by far the most abundant species in the field. Eggs were collected until November 4, when snow halted test work. This fly was most numerous during the latter part of September with a peak of 307.4 eggs per fruit deposited on September 29. Eggs of *Drosophila simulans* were first collected on October 21. Apparently they were late-comers to tomatoes, possibly having been on other food materials not available late in the season.

FRUIT PREFERENCE.—Tests were conducted in 1952 to determine at which stage of ripeness the tomatoes were most attractive to the flies for egg deposition. On September 17 tomatoes were collected and cut in the same manner as for the population determination studies. Five different stages of ripeness were arbitrarily chosen on the basis of color. The cut fruits were placed in strawberry boxes, two of one stage of ripeness in each box. The boxes were placed in the tomato row and replicated six times. The results, shown in table 1, indicate that eggs were deposited on fruits of all stages of ripeness, with fruits of the third stage being the most attractive. Since these fruits were several days from canning ripeness, these observations indicated that canning ripeness tomatoes were not the preferred media for egg deposition. No record was made of the species of *Drosophila* eggs deposited.

In 1953 several tests were conducted in an attempt to confirm the observations of the previous year. Only three stages of ripeness were used. Three separate tests were conducted and each time the stages of ripeness were matched by means of kodachrome pictures. The tomatoes of medium ripeness were considered to be from 3 to 4 days from canning ripeness. The results obtained are shown in table 2. These tests indicated that once the tomatoes had

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Table 1.—*Drosophila* egg deposition on tomatoes of different stages of ripeness. 1952.

STAGE OF RIPENESS	TOTAL EGGS DEPOSITED	AVERAGE OF 12 REPLICATES
1. Red ripeness	315	26.3
2. Medium red	409	34.1
3. Medium ripeness	448	37.3
4. Pink	197	16.4
5. Green	26	2.2

Table 2.—*Drosophila* egg deposition on tomatoes of different stages of ripeness. 1953.

Stage of Ripeness	Egg Deposition	
	Total	Average
September 9–10 (8 replicates)		
Red ripeness	367	45.9
Medium ripeness	262	32.8
Green	92	11.5
September 11–12 (12 replicates)		
Red ripeness	575	47.9
Medium ripeness	971	80.9
Green	128	10.7
September 23–24 (12 replicates)		
Red ripeness	698	58.2
Medium ripeness	502	41.8
Green	215	17.9
Totals		
Red ripeness	1640	51.3
Medium ripeness	1735	54.3
Green	435	13.6

turned color, there is not much difference in attractiveness for egg deposition.

Species Observed at the Canning Factory.—Ditman & Bickley (1952) reported that most of the flies found at the canning factory in the late season were *D. melanogaster*. About 10% of the flies present were *D. hydei*. In the fall of 1952 observations were made at the Campbell Soup Company's yard at Camden, New Jersey. The *Drosophila* problem was very acute and tremendous numbers of flies were present on the loads of tomatoes. The company was using a Dyna-fog machine in their control operations and large accumulations of dead *Drosophila* were on the ground, a large number of which were brought back to the laboratory for identification. Identifications of the species involved were confirmed by Dr. W. W. Wirth of the Entomology Research Branch, U. S. Department of Agriculture. A sample of 1635 flies was taken from those collected for identification. The results, shown in table 3, showed *D. melanogaster* was the most abundant species, comprising 78.2% of the flies sampled. More males than females of *D. melanogaster* were found, a fact that may be explained by one or more of the following: (1) there could have been more males than females present, (2) most of the females were located on the lower layers of fruit, whereas the males were on the tops of the loads, and (3) the males are usually more susceptible to insecticides than the females and the sample was of dead insects.

Table 3.—Species of *Drosophila* collected at canning factory, Camden, New Jersey, Fall, 1952.

Species	Number of Flies	Per Cent of Total
D. melanogaster	1278	78.2
males	798	48.9
females	480	29.4
D. busckii	188	11.5
D. tripunctata	33	2.0
D. hydei	32	2.0
D. immigrans	9	0.6
Damaged specimens	95	5.7
Total flies examined	1635	100

Observations on Control.—The control of *Drosophila* in cannery tomatoes has many aspects. Examination of tomato loads as they arrived at the factory showed heavy infestation with flies, eggs, and to some extent with recently hatched maggots. As the tomatoes were moved many times between the time of picking and the time of arrival at the canning factory, many were cracked and crushed and thus open for egg deposition. Often an interval of several hours occurred between arrival and unloading during which the tomatoes were heavily attacked by the flies.

Ditman *et al.* (1936) attempted to control *Drosophila* by using traps and insecticides. Although the traps gave no apparent reduction in the population, a pyrethrum spray was effective. Armitage (1946) reported good control with residual spraying of DDT, and Sampson (1950) recommended residual spraying with DDT and chlordane. Starnes (1953) recommended the use of alcoholic extracts of pyrethrum with wetting agents added for the control of flies on loads. Pepper *et al.* (1953) recommended the use of mist blowers and fog generators with pyrethrum as the insecticide. Bickley & Ditman (1953) recommended the use of electric atomizing sprayers as superior to hydraulic sprayers.

Fog Machine Tests on Truckloads of Tomatoes.—In the latter part of September, 1952 observations and experiments were conducted at the Campbell Soup Company's processing plant in Camden, N. J. Efforts were being made at the factory to reduce or control the fly populations on the trucks with the use of a Dyna-fog machine. The trucks were driven into a canvas semi-enclosure where the fogging took place. Approximately 1 quart of a pyrethrum-piperonyl butoxide mixture was used on each truckload of tomatoes. The penetration of smoke through and over the truck appeared to be excellent. It was apparent, however, that the penetration through each individual basket was poor. Thermocouple readings taken at the mouth of the machine showed that temperatures of 225 to 400° C. occurred during the fogging process.

Prior to the test fogging, small cages containing *Drosophila* were placed in various locations of the load. The cages were made from two Mason jar lids with a fine-mesh, organdy cloth glued over the open tops, and the two lids held together with masking tape. During the fogging treatment, flies on the wing in the fog were netted. Flies were also netted before the fogging and caged to determine the natural mortality. Each truck, containing 250 to 300 baskets of tomatoes, was fogged for 30 to 45 seconds using about 1 quart of material per load.

The results of the fogging tests are shown in table 4. The control obtained from the first three formulations listed was very low. The fogging with 3% DDT was also very disappointing. With the 2% lindane, however, 100% control was obtained. With the materials other than lindane, higher kills were obtained on the side of the truck nearest to the fogging machine.

Residue analyses were made of samples of tomatoes from the DDT and lindane treatments. The methods used for DDT analysis were total chlorine and colorimetric

Table 4.—Effectiveness of several formulations against *Drosophila* on truckloads of tomatoes when applied with a Dyna-fog machine.

Insecticide Treatment	Cages	One-Hour Mortality			Two-Hour Mortality	
		Dead	Total	Per Cent Kill	Dead	Per Cent Kill
Pyrethrum 0.2% Piperonyl butoxide 1.69%	10	95	418	22.7	201	48.1
Allethrin 1.67% Butoxy polypropylene glycol 3.33%	10	34	1038	3.3	171	16.5
Allethrin 0.4% Piperonyl butoxide 1.67%	10	60	332	18.1	118	35.5
DDT 3%	6	13	236	5.5	58	24.6
Lindane 2%	6	162	162	100	162	100
Control		6	402	1.5	46	11.4

and for lindane total chlorine. Although the baskets from which the samples were taken were from the part of the truck nearest the fogging machine, no insecticide residues were found.

FIELD CONTROL.—It is of prime importance that a successful control be developed for the flies in the field. Pyrethrum dust (Ditman *et al.* 1936) and DDT emulsion (Ditman & Bickley 1952) are ineffective. Lindane emulsion gave 78% reduction over 8 days, but because of possible off-flavor difficulties, it was not recommended (Ditman & Bickley 1952). Although Warner (1951) obtained good kill of *Drosophila* in fig orchards with 2% TEPP dust applied by aircraft, the control was only of short duration. Michelbacher *et al.* (1953) showed that a 10% DDT dust and a dieldrin spray using 1 lb. actual per acre were effective.

During 1952, at the Campbell Soup Company's farm at Riverton, N. J., *Drosophila* activity was observed in a field of tomatoes which had been sprayed with a number of materials for insect and disease control. A series of 11 applications were made at 10- to 11-day intervals during the season on replicated plots, four rows wide by approximately 50 feet in length. The graded fruit from each picking was processed into juice and egg and maggot counts of *Drosophila* were made according to the procedure described in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1945) The results of these studies are shown in table 5. The data obtained from the untreated samples of juice had considerably higher counts than any of the treatments. The most effective control was with TDE which gave 78.2% reduction in the number of eggs when compared with the untreated sample.

SUMMARY.—Observations in tomato fields in New Jersey indicated the presence of *Drosophila melanogaster*, *D. tripunctata*, *D. simulans* and *Scaptomyza adusta* and the first named species was the most abundant during the tomato harvesting season. Largest numbers of eggs were deposited during September in 1952 and 1953. The flies deposited eggs in tomatoes of all stages of ripeness with little or no preference after the tomatoes have once turned color. The flies present at the canning factory were primarily *D. melanogaster*, with *D. busckii* the second most abundant species.

Tests were made of the effectiveness of Dyna-fog applications of pyrethrum and allethrin formulations, DDT, and lindane for the control of *Drosophila* on truckloads of tomatoes. Lindane appeared to offer the most promise. The number of eggs and maggots of *Drosophila* varied in tomatoes canned from plots treated with different insecticides. Of the materials tested, TDE gave the largest reduction in *Drosophila* contamination.

Table 5.—The effect of various treatments applied to tomato plants on the number of eggs and maggots of *Drosophila* appearing in the processed juice.

Treatment	Egg and Maggot Counts for Various Picking Dates[a]									
	8/4	8/12	8/18	8/25	9/1	9/8	9/15	9/22	9/29	Total[b]
DDT (tribasic copper+ziram)	0	0	0	2	0	2	0	4	10	18
TDE	0	0	0	0	2	0	0	8	2	12
Methoxychlor	0	0	0	2	0	0	0	4	12	18
Calcium arsenate+parathion	0	0	0	0	0	0	0	18	4	22
DDT+parathion	0	0	0	0	2	0	0	17	1	20
Calcium arsenate	0	0	0	0	0	1	0	13	9	23
DDT (manzate)	0	0	0	2	0	0	2	22	9	35
Untreated	0	0	0	6	0	0	4	40	5	55
Dates of treatments		8/7	8/18	8/28	9/8					

[a] Counts made on total contents of No. 2 can.
[b] Total count from nine No. 2 cans.

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The Effectiveness of Pyrethrum and Allethrin Formulations in a Thermal Fog Machine[1,2]

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Brown & Morrison (1954) have described the assessment of a *Swingfog* portable jet apparatus which appeared to have promise in insect control operations. On food products where the accepted limit of insecticide residue may be quite low, use of non-residual or low residual type materials, such as pyrethrum or pyrethrum-like materials may be advisable. The use of these materials in fogging machines has raised the question of the possible breakdown or loss of effectiveness of the insecticide as a result of the fogging operation. Tests were therefore designed to ascertain the possible breakdown of formulations of pyrethrum and allethrin when used in a *Swingfog* machine.

METHODS AND PROCEDURES.—Formulations were prepared in the laboratory and stored under refrigeration until time of use. D & C Violet No. 2 dye (Pylam Products Company Inc., 799 Greenwich Street, New York City) was included in all formulations so that evaluations could be made later. Perchlorethylene was the solvent used in all of the tests. Concentrations of the insecticides were calculated as grams per 100 cubic centimeters and were recorded as per cent.

The fog machine was set to deliver a wet fog. An earlier test with a thermocouple placed at the end of the delivery tube 2 inches from where the insecticide is introduced indicated that the machine ran cooler at this setting. When running dry, that is producing no fog at all, the temperature was approximately 625° C. When delivering a fairly dry fog, the temperature was 575° C. When delivering a wet fog, the temperature was 525° C. It was decided to use a wet fog since the breakdown, if any, of the materials at this lower temperature would most probably be as much if not greater than with a dry fog or with smaller droplets.

A modified vacuum cleaner was used to collect the samples. A plastic cup was placed at the end of the vacuum cleaner hose in which a glass wool filter collected the insecticide from the fog passing into the hose. The filter was placed in 50 cubic centimeters of acetone which removed the collected materials from the filter. The samples were collected by holding the cup on the end of the vacuum clearner hose about 1 foot from the end of the fog mixing tube until 100 linear feet of air had passed through the cleaner. This was measured by means of a manometer placed in the exhaust section of the vacuum cleaner.

The samples were stored under refrigeration until they were evaluated. The concentration of the material was determined by the use of a Beckman spectrophotometer, with the D & C Violet No. 2 dye as the indicator. The wavelength was 575 millimicrons and the slit opening was 0.06 millimeter. The collected samples were simultaneously compared with the original stock solutions. The concentration of the collected sample was calculated by comparing the optical density.

Third instar larvae of *Aedes aegypti* (L.) were used as the test insect to determine if the insecticidal activity had been affected by passage through the machine. A dilution series was run simultaneously with the stock solution which had not been fogged. Counts were made of the dead mosquito larvae after 24 and 48 hours. The criterion for death was the inability of the larvae to move after the beaker in which they were contained was tapped with a pencil and held over a strong light. Water and acetone checks were also run simultaneously. Three replicates of each concentration were run for 1 day and the test repeated once to give a total of six tests and approximately 120 larvae per concentration.

[1] *Swingfog* machine provided by Devenco Incorporated, New York, N. Y.

[2] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Entomology, New Brunswick. Accepted for publication February 9, 1956.

[3] Presently in the United States Army.

Table 1.— Toxicity of pyrethrum formulations to larvae of *Aedes aegypti* before and after having been put through a *Swingfog* insecticide generator.

Test Material	Before Fogging			After Fogging		
		Per Cent Kill			Per Cent Kill	
	P.p.m.	24-Hour	48-Hour	P.p.m.	24-Hour	48-Hour
Pyrethrum 0.5%	0.005	4.9	4.9	0.005	1.6	3.3
	.01	20	22.4	.01	15	16.7
	.02	83.7	87	.02	50.8	57.1
	.04	100	100	.04	90	95
	.08	100	100	.08	100	100
Pyrethrum 0.5%+ piperonyl butoxide 4.0%	.005	27.1	35.3	.005	30.8	37.6
	.01	79.6	89.1	.01	86.5	95.5
	.02	100	100	.02	100	100
	.04	100	100	.04	100	100
	.08	100	100	.08	100	100
Pyrethrum 0.5%+ sulfoxide 4.0%	.005	5.9	11.1	.005	10.7	13.2
	.01	52.0	74.4	.01	42.1	73.1
	.02	100	100	.02	100	100
	.04	100	100	.04	100	100
	.08	100	100	.08	100	100
Pyrethrum 0.5%+ n-propyl isome 4.0%	.005	2.6	5.2	.005	1.8	7.3
	.01	32.0	60	.01	19.9	40.7
	.02	100	100	.02	85.8	96.5
	.04	100	100	.04	100	100

RESULTS.—The bioassay tests were conducted against the larvae on a total of 10 separate days. Since the toxicity to the larvae from distilled water and acetone was low, these were ignored in the analysis of variance. The average mortality from distilled water was 0.16% at the 24-hour count and 0.33% mortality at the 48-hour count. With acetone there was 0.33% mortality at the 24-hour count and 0.83% mortality at the 48-hour count. A filter was soaked in 50 milliliters of acetone as were the test filters. An aliquot of 0.5 ml. was used and an average of 1.62% mortality at the 24-hour count and 3.26% at the 48-hour count was obtained. Since a large aliquot was used and the resulting mortality was low, this was omitted in the analysis of variance.

Table 2.—Toxicity of allethrin formulations to larvae of *Aedes aegypti* before and after having been put through a *Swingfog* insecticide generator.

Test Material	Before Fogging			After Fogging		
		Per Cent Kill			Per Cent Kill	
	P.p.m.	24-Hour	48-Hour	P.p.m.	24-Hour	48-Hour
Allethrin 0.5%	0.01	0.9	0.9	0.01	0	0
	.02	1.7	3.4	.02	0	0
	.04	9.5	10.4	.04	0	3.4
	.08	58.3	65.8	.08	15.4	18.8
	.16	96.6	99.1	.16	89.9	94.9
Allethrin 0.5%+ piperonyl butoxide 4.0%	.005	0	0.8	.005	0	0.8
	.01	0	0.9	.01	0	0
	.02	.8	1.6	.02	2.5	3.3
	.04	14.0	26.5	.04	4.1	5.8
	.08	89.2	96.7	.08	37.8	46.2
Allethrin 0.5%+ sulfoxide 4.0%	.005	.8	0.8	.005	0	0
	.01	2.5	4.2	.01	2.5	2.5
	.02	22.7	45.4	.02	10.8	18.3
	.04	48.3	66.7	.04	50.8	66.1
	.08	99.2	100	.08	98.3	100
Allethrin 0.5%+ n-propyl isome 4.0%	.005	.8	0.8	.005	0	0
	.01	.8	3.3	.01	0	0
	.02	18.5	38.7	.02	0	2.5
	.04	47.5	72.1	.04	14.0	24.8
	.08	98.3	100	.08	67.8	75.2
Allethrin 0.5%+ MGK 264 4.0%	.005	.8	0.8	.005	0	1.7
	.01	.8	0.8	.01	0	0
	.02	13.6	17.8	.02	0	.8
	.04	44.5	47.1	.04	27.5	33.3
	.08	88.2	94.9	.08	68.3	73.3

The results of tests involving the use of pyrethrum and pyrethrum in combination with several synergists are shown in table 1. Analysis of variance was conducted to determine the loss, if any, of toxicity. With pyrethrum alone, there was a highly significant difference between the pre- and post-fogged samples; the fogging decreased the effectiveness of the toxicant. Pyrethrum plus piperonyl butoxide and pyrethrum plus sulfoxide showed no significant difference between the pre- and post-fogging. The results of pyrethrum plus n-propyl isome indicated that at the 48-hour count there was a significant loss in toxicity.

The results of tests involving allethrin and allethrin in combination with four different synergists are shown in table 2. With allethrin alone, there was a highly significant reduction after fogging. Allethrin plus piperonyl butoxide and allethrin plus n-propyl isome also showed highly significant differences between pre- and post-fogging tests. No significant difference occurred with the allethrin plus sulfoxide formulation. Allethrin plus MGK 264 showed no significant difference at the 48-hour count.

DISCUSSION.—Tests indicated that pyrethrum and allethrin in the absence of synergists, allethrin plus piperonyl butoxide, and allethrin plus n-propyl isome will show highly significant losses in toxicity after being put through a *Swingfog* insecticide applicator.

It is interesting to note that piperonyl butoxide plus pyrethrum withstood the fogging whereas piperonyl butoxide plus allethrin did not. This would appear to indicate that a better synergist is needed for allethrin formulations. Sulfoxide formulations of pyrethrum and allethrin were quite stable. Although the mechanics of this protection is not known, it would appear that sulfoxide is an effective synergist when the materials are to be put through a thermal fogging machine. It is somewhat surprising that, as a rule, the pyrethrum formulations withstood the fogging better than those involving allethrin.

Since a wet fog was used and the machine ran cooler at this setting, there is the possibility that the materials which showed no loss in toxicity under the conditions used would show a loss when a dryer fog was used and the temperature was higher. It is thought, however, that the materials that showed a significant loss in toxicity in this test would also do so under more severe conditions.

SUMMARY.—Tests were conducted to determine the loss in toxicity of formulations of pyrethrum and allethrin when passed through a *Swingfog* insecticide applicator. Samples of the fog were collected and tested against formulations not subjected to the fogging process. Larvae of *Aedes aegypti* (L.) were used as the test insect. Under the conditions tested, pyrethrum, allethrin, allethrin plus piperonyl butoxide, and allethrin plus n-propyl isome showed highly significant losses in toxicity as a result of the fogging treatment.

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Sampling Studies Related to Insecticide Residues on Cabbage and Celery[1]

C. H. Van Middelem,[2] J. W. Wilson,[3] and W. D. Hanson[4]

No analytical result can be better than the sample it evaluates. Sampling, therefore, should be considered as an integral part of the insecticide-residue analytical procedure. If sampling is inadequate or improper, the time spent on costly chemical analyses is utterly wasted; the conclusions drawn will be erroneous.

Therefore, it is important that the entomologist and chemist discuss and approve the proposed experiment before the vegetable to be studied for residues is planted. To achieve best results, the chemist must be given ample opportunity to consider the following factors: practicability and feasibility of the analytical method, total number of samples to be taken, field and sub-sampling procedures, extraction technique and the availability of cold storage facilities for sample extracts.

Although numerous publications have reported on analyses of pesticide residues, only a limited number mentioned the sampling procedure used; a still smaller number attempted to evaluate the variations involved in their sampling technique. A rather complete digest of the literature, from 1925 through 1952, related to sampling of crops for spray residue analyses, has been prepared by Fahey (1954). In this compilation, two articles relate to sampling of cabbage and none to celery.

Insecticide chemists are often confronted with unsatisfactory reproducibility and erratic results in analyzing extracted residue samples. In looking over the disappointing data, the analyst might feel that his technique is subpar. This, of course, might be part of the answer.

However, when dealing with leafy crops, the chances are very good that a considerable amount of the variation can be traced to the sampling procedure followed in the field and the subsequent choice of a subsample for solvent "stripping." When one considers the heterogeneity of the plant surface area, possible variations in application, differences in plant size and the human failure to sample randomly without bias in the field, it is obvious that sampling variation is one of the paramount sources of erratic results in the laboratory.

The amount of freshly harvested vegetable material to be selected for residue analyses can be estimated by statistical analysis if a measure of the variability has been obtained. According to Gore (1952), the primary basis of statistical estimation of experimental errors is precision or reproducibility. Unfortunately this is quite different from accuracy. A measurement may be extremely variable; but if, as more determinations are made, the average begins to approach the true value as a limit, the measurement may be said to be accurate—but not necessarily precise.

Gore (1952) also states that another frequent cause of bias in experimental designs is the failure to obtain an adequately representative sample of the materials on which the tests are performed. There are two requirements for satisfactory sampling: (1) *The sample must be valid.* Validity is achieved by selecting the sample in a manner which insures that each unit of material in the batch being sampled has an equal chance of being chosen for the extraction and ultimate test. This process is known as randomization, and a sample taken in this manner is considered a random sample. (2) *The sample must be representative.* A representative sample is not only a random sample but the proportion of each type of plant material in the celery or cabbage sample is identical with that of the gross sample from which it was selected. Of course, no sample can be absolutely representative. A common criterion for a representative sample is that it should be of such size and composition that, if a series of such samples were taken from the population, sample-to-sample variations would be insignificant, compared to the errors of measurement.

Usually, the more plants randomly sampled in a field plot, the more representative the sample becomes. However, the economics involved will usually dictate the magnitude of the sampling program for residue studies. As stated by Gunther & Blinn (1955), the investigator in many instances must rely on his good judgment rather than upon established sampling procedures when working with certain crops.

Understandably, the variation to be expected in sampling a leafy crop such as celery or cabbage for residues would be much greater than the variation found in a sampling study on apples or oranges, because of the tremendously greater heterogeneous surface area involved. Because of this factor, the residue would be deposited on the leafy crop in a non-uniform manner. The task of obtaining a valid, representative sample of a celery plot is therefore much more laborious and difficult than getting a similar apple or orange sample.

Assuming that a valid, representative sample has been chosen and extracted, it would appear relatively simple to apply the approved chemical method and run the analysis. Unfortunately, this is often not the case. A particular method that has been successfully worked out on one crop in one section of the country may require considerable modification on another crop grown in another area. Many of the common procedures have been tested extensively on certain fruit, but considerable difficulties arise when these same procedures are tried on leafy vegetables. The presence of waxes, oils, terpenes, pigments, aldehydes, organic acids, and other extraneous extracted matter often causes serious interferences in the final colorimetric phase.

Since most of our modern residue chemistry has been developed in the past decade, many steps in new procedures have not been verified on extracts taken from all the common vegetables. Therefore, each method has to be more or less redeveloped on every new crop on which it is tested. In addition, not only are these analyses very recent

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in their development, but the insecticide molecule (or part thereof) for which the method is specific is often rather complicated and subject to numerous chemical changes under certain conditions. Moreover, concentrations of insecticide residues down to as low as 0.1 part per million or lower are detectable with many of the newer methods; this results in even more apparent variation.

It was with some of these problems in mind that the field sampling evaluations on celery and cabbage were designed and supplemented by the laboratory procedural variation study.

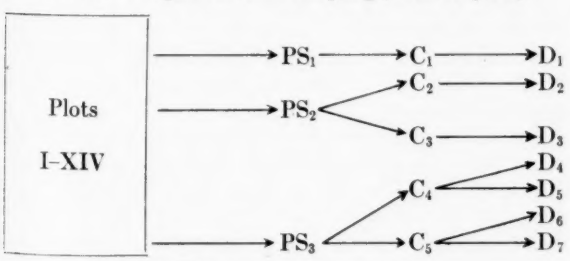
MATERIALS AND METHODS.—In April, 1954, a mature field of celery was sprayed uniformly with 25% parathion wettable powder, at the rate of 2 lbs. per acre. A power sprayer was used, operating at 200 pounds per square inch pressure and was driven at a speed which would allow application of 90 gallons spray per acre. The sprayer was equipped with five cone-type nozzles per row: one was directly over the row; one on each side was directed downward at an angle of 45° from horizontal; and one on each side of the row was parallel to the ground and about 8 inches from ground level.

In March 1955, 0.5 lb. of technical parathion per acre, formulated as a 50% liquid concentrate, was applied to a mature field of cabbage. The same spray equipment and gallonage was used as in the previous year on celery. Each experiment was composed of 14 four-row plots, 65 feet long. Both experiments had one uniform insecticide application and all field samples were taken between 2 and 3 hours later. The plants were relatively dry when sprayed and the insecticide was allowed to dry on the plants before sampling began.

Sampling.—The sampling scheme illustrated in fig. 1 was used in both the celery and cabbage experiments. This use of unequal sub-classes reduced the number of necessary laboratory determinations by approximately 40% without sacrificing excessively the information required for proper statistical interpretation. Three separate plant samples (PS No. 1, 2, and 3), composed of 10 stalks or heads each, were cut at random from each of the two center rows of all 14 plots. Care was taken to avoid choosing plants from the 6-foot space at the ends of each row. Each plant sample was placed in a burlap bag and immediately brought to the laboratory for processing. Samples were kept at 40° F. until cutting and extraction could be accomplished.

Schematically, fig. 1 illustrates the manner in which one 300-ml. aliquot was saved for laboratory analysis (D_1) from the sub-sample (C_1) extracted from plant material taken from plant sample No. 1 (PS_1). Only one 300-ml. aliquot was saved for analysis (D_2, D_3) from each of the two sub-samples (C_2, C_3) extracted from material taken from plant sample No. 2 (PS_2). However, from each of the two extractions made of sub-samples (C_4, C_5) taken from plant sample No. 3 (PS_3), two 300-ml. aliquots (D_4, D_5, and D_6, D_7) were saved for analysis. Consequently, three separate 10-plant samples, five sub-samples were saved from each plot. From the five sub-samples, a total of seven laboratory determinations were conducted per field plot.

FIG. 1.—Unequal sub-class sampling (Total 14 plots)



PS = Plant samples (10 plants each).
C = Sub-sample saved for extraction.
D = Laboratory determinations.

Sub-Sampling—Celery.—Each of the 10 mature celery plants (plant sample) were field trimmed, cut into 1-inch segments, placed in a rotary drum and well-mixed. Approximately 4 inches of the celery butt was discarded. From the mixed plant sample, a 1,200-gram representative sub-sample was chosen at random for 30 minutes of extraction with 1,000 ml. benzene.

Sub-Sampling—Cabbage.—The cabbage plant samples (composed of 10 mature heads), were harvested in the usual commercial manner, saving the standard number of wrapper leaves. Each marketable head was quartered and 5 to 6 layers of the outer leaves were composited from each quarter. All of the composited leaves from each plant sample of 10 heads were well-mixed in a rotary drum and a 1,000-gram representative sub-sample was chosen for extraction with 1,000 ml. of hexane.

After extraction, the aliquot "strippings" were placed in sealed pint Mason jars and placed in 40° F. storage until analyzed. The celery benzene extracts were analyzed within 2 weeks of the date of extraction. The cabbage hexane extracts were analyzed between 6 and 8 weeks after the extraction date.

RESULTS.—Two previous laboratory experiments reported by Sharpe *et al.* (1955) had elucidated the more critical steps of the parathion analytical procedure as modified by Gunther & Blinn (1950). The results of these tests, with the determination errors from the field sampling experiment, are summarized in table 1. In the preliminary study, it was found that the largest source of error resulted from the reduction step, and that the analytical procedure was highly reproducible when pure benzene was used. Furthermore, it was noted that the determination error was substantially increased by the introduction of plant extracts as compared to the precision with pure benzene. Variability apparently was not associated with individual plant sources. In the field sam-

Table 1.—Estimate of variance components associated with the laboratory determination of parathion.

VARIANCE COMPONENT	LABORATORY TESTS		CELERY FIELD SAMPLING	CABBAGE FIELD SAMPLING
	Benzene	Celery Extraction		
Determination	*5.90*	*39.9*	*102*	*260*
Days	0.0	5.9		
Plant extracts	—	0.0		
Evaporation	5.4	8.4		
Reduction		24.4		
Readings	0.6	1.2		

Table 2.—Estimate of variance components associated with the field sampling of celery.

Variance Component	Celery		Cabbage	
	Value	Per Cent	Value	Per Cent
Plot	151[a]	36.0	102	17.7
Plant sampling	144[a]	34.3	184[a]	31.9
Cutting and extraction	23	5.4	31	5.4
Determinations	102[a]	24.3	260[a]	45.0
Total	420	100.0	577	100.0

[a] Different from zero based on at least .05 level of probability.

pling study with celery, the determination error was estimated to be 102 (table 1), as compared with the comparable value of 39.9 obtained in the previous study. This difference could be attributed to the source of celery, since the celery in the preliminary study was purchased at a local store and had been shipped from a northern market.

The variance components determined in both the celery and cabbage experiments were as follows: (1) plot; (2) plant; (3) cutting and extraction; (4) laboratory determinations. Table 2 shows the value of the estimated variance and the per cent of the total variability attributable to each of the various components under study.

Based on the actual estimated costs of each phase of this study, table 3 gives the relative percentage costs. The figures are the percentage of the variable cost for a determination associated with each step:

Table 3.—Percentage costs of sampling phases per laboratory determination.

Phase	Component	Variable Costs (Excluding Fixed Costs)
Plots	σB^2	60%
Plant samples	σP^2	8%
Cuttings and extractions	σC^2	15%
Determinations	σD^2	17%

The difference between the cost of setting up and handling the first plot plant sample, cutting plus extraction and the laboratory determination, and that of handling an additional plot plant sample, etc., is defined as fixed costs. The assumption, therefore, is made that the experiment will be run.

Consequently, based on the data in table 1 and with cost estimates of the various procedures of sampling and determination, the optimum allocation for sampling in parathion residue studies is given in table 4.

Table 4.—Optimum allocation for sampling parathion residues.

Crop	Plant Samples[a] per Plot	Cuttings per Composite	Determinations per Aliquot
Celery	3	1	2
Cabbage	4	1	3

[a] Composed of 10 randomly chosen mature plants.

Discussion.—The total variability in the cabbage experiment was higher than that of the celery test. Of the four major sources of variation studied, the cutting and extraction phase of the sampling study on both crops remained consistently low. The plant-sampling error for both celery and cabbage contributed approximately one-third of the total variation. However, plot-to-plot variation for celery was twice as high percentagewise as was the cabbage-plot error. This difference might be partially explained by the fact that the celery plant has somewhat more of a heterogeneous surface than the mature cabbage head. Therefore, more error in plot-to-plot sampling would be expected. Perhaps the weather was not as suitable for uniform insecticide distribution on the day the celery was sprayed.

In the cabbage study, the determination error was considerably higher than it was for celery. This could be explained partially by the greater difficulty experienced in the laboratory during the "clean-up" phase of the cabbage extracts due to the presence of varying quantities of waxes, oils, terphenes, etc. These extraneous materials are extracted simultaneously with the insecticide. If not eliminated, they will result in interferences during the final colorimetric steps of the procedures. The determination percentages for both cabbage and celery may seem excessive, but these figures are relative and merely percentages of the total variation experienced from the field plot to the final laboratory determinations.

It is interesting to compare the increasing relative analytical errors in the parathion procedure beginning with pure benzene and following it through the celery and cabbage field experiments. These striking differences reemphasize the point stressed earlier that a procedure tested originally on a relatively clean extract from a smooth-surfaced fruit only, will in many instances, confront the chemist with much greater difficulties when tried on an extract from a waxy, leafy vegetable.

As a result of the laboratory tests on celery extracts, it was shown that the reduction step alone in the analytical procedure contributed over 60% of the total determination error. When the evaporation and reduction steps were combined, over 80% of the total determination error was involved.

An attempt was made to estimate the relative percentage costs for each phase of study on the two crops. Exclusive of fixed costs, it was estimated that the variable costs associated with a single plot—such as plowing, hoeing, cultivating, planting, and spraying—would account for approximately 60% of the total variable cost of one laboratory residue determination. This is based on the fact that the field experiment was designed solely for insecticide residue studies. The chemist's time, cost of chemicals, equipment, etc., and the time and solvents involved in the cutting and extraction phase accounted for approximately one-third of the total variable cost.

Size of the field plot and economics may not permit the sampling of 30 celery plants and 35 to 40 cabbage heads. If it is felt that the cost of taking a plant sample of cabbage is proportionately greater than for celery, then 25 to 30 heads might suffice. One well-mixed 1,000-gram subsample per composite is sufficient for both vegetables. Two determinations are satisfactory for celery, but it would appear that three will be required of the cabbage

extract because of the relative size of the determination-component error.

SUMMARY AND CONCLUSIONS.—The determination-error value for cabbage was over twice as high as it was for celery. Expressed in percentage, the laboratory error accounted for 24.3 and 45.0% of the total error in the celery and cabbage tests, respectively. The cutting and extraction error was relatively insignificant in both experiments. The plant-to-plant and plot-to-plot per cent of the total variation for celery was 34.3 and 36.0, respectively. The same two sources of error on cabbage were respectively, 31.9 and 17.7%.

It was found that the determination errors progressively increased from the first test—where parathion was merely added to pure benzene—to succeeding experiments using celery and cabbage extracts taken from field-sprayed samples.

Estimated plot costs account for 60% of the total variable costs involved in obtaining one laboratory determination. Cutting, extraction, and the actual laboratory procedures resulted in approximately 32% of the total variable costs of one analysis. Obtaining the plant samples in the field accounted for about 8% of the total variable cost.

Additional effort, as a result of the source of variation study, will be made in an attempt to reduce the determination error in the parathion procedure. Preliminary laboratory tests indicate that the reduction and evaporation steps contribute most of the total analytical error. Therefore, future studies will be concentrated on these critical steps.

The use of variance components in two sampling studies of parathion residues has made possible the following recommendations if plot size and economics permit: (1) sample at random 30 celery plants and 35 to 40 cabbage heads per plot; (2) cut, composite and draw one sample for extraction; (3) run duplicate determinations for celery and triplicate analyses for cabbage on each composite extraction; (4) replicate as much as total costs will allow or to achieve the desired precision if costs are not a limiting factor.

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Digger Wasps, *Tachysphex* spp., as Predators of a Range Grasshopper in Idaho[1]

R. C. NEWTON,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

In 1953 digger wasps preying on grasshoppers near Orovada, Nevada, were reported by personnel of the Plant Pest Control Branch. On June 2, 1954, Frank T. Cowan and Lee Seaton observed many medium-sized digger wasps with orange abdomens stinging and burying large numbers of nymphs of the grasshopper *Oedaleonotus enigma* (Scudder), a range species, about 18 miles east of Shoshone, Idaho. Some of the species collected in the area were identified as *Tachysphex tenuipunctus* Fox.[3] In the collections there were also at least 10 unnamed species of the same genus. *T. tenuipunctus* and two of the unnamed species, which were preying on nymphs of *enigma*, are considered in the following discussion.

As early as 1878 Riley *et al.* (1878) reported and illustrated a small wasp "with black head and thorax and reddish abdomen, while in the act of carrying a *spretus* larva." It was identified as *Larrada semirufa* Cresson. Two years later (1880) they reported on specimens of a slightly larger digger wasp, identified as *Larra tarsata* Say, that Riley had received from W. C. Lemmon, Sierra Valley, California. Regarding this second wasp, Lemmon wrote "We have (to us) a locust exterminator, that in certain localities kills and buries large numbers. It appears to kill the locust by stinging him, thrusting into his body an egg which hatches in a few days into a little grub." A few days later Lemmon again wrote regarding this wasp, "I hear much and see a few specimens of a species of *Pompilus* which has been very active in catching, stinging, and dragging locusts into holes dug in the sand, and covering them hastily—then seize new victims in rapid succession."

The generic name of both wasps mentioned in Riley's papers was later changed by Fox (1893) to *Tachysphex*. Thirty years later in Kansas Williams (1913) studied the habits of a number of adults of this genus, including *fusus* Fox, *terminatus* Smith, *plenoculiformis* Williams, *propinquus* Viereck, and *tarsatus* Say. Williams (1945) also published an account of the life history of *T. fanuiensis* Chessman preying on a cockroach in New Caledonia.

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[2] The author wishes to acknowledge the valuable assistance of Lee Seaton, now with the Plant Pest Control Branch, in carrying out part of this study.

[3] Determinations were made by K. V. Krombein, Entomology Research Branch.

The habits of the digger wasps observed in Idaho in 1954 closely resemble those of the wasps described by Lemmon, and a number of the species, including *tarsatus*, described by Williams. The present studies give additional information on the larvae and pupae of three species of the genus *Tachysphex*.

ABUNDANCE AND ECONOMIC IMPORTANCE.—Observations were made on these species over an area 4 miles long and ½ mile wide from 14 to 18 miles east of Shoshone during most of June 1954. When the wasps were first discovered, most of the host nymphs were in the second to fifth instars. The nymphs tended to migrate in concentrated bands of 20 to 30 per square yard. The wasps were stinging and burying the nymphs in the sandy soil. Usually they placed 2 nymphs in each hole. At one place the examination of 1 square foot of soil uncovered 8 grasshopper nymphs, 4 wasp cocoons, and 3 dipterous parasites, representing at least 13 grasshoppers that had been killed and buried. In another area of the same size 22 paralyzed or dead nymphs of *enigma* were found. In addition, as many as six paralyzed nymphs per square yard were left by the wasps on the soil surface.

By mid-June *enigma* grasshoppers were reduced to five per square yard, and most of them were in the third to sixth instar, although an occasional adult was present. Wasps were burying only one grasshopper per hole, owing possibly to the larger size. Examination and sifting of soil samples at selected places on the range showed about four buried nymphs per square foot. By June 23 the grasshopper population was further reduced to an average of one in 5 square yards. The reduction over the entire area was due to the constant digger wasp activity.

The area where these studies were made constituted a small portion of an infestation of *enigma* covering about 200,000 acres of rangeland. Plans had been made to spray the entire infestation by aircraft. Digger wasps were widely distributed and very active, and it is believed that drastic reductions occurred in *enigma* over the whole territory concomitantly with the reductions on the study area. By June 16 the infestation that needed control was reduced by at least 75,000 acres. No sprays were applied to the study area in 1954, but large numbers of wasps emerged in June 1955, and they had virtually no grasshoppers on which to prey.

DESCRIPTION.—*Adult.*—The adult wasps range from 10 to 16 mm. and average about 13 mm. in length; they have a wing spread of about 18 mm. The head and thorax are dull black (fig. 1). In *tenuipunctus* and one of the undetermined species the posterior one-third of the abdomen shades into a dull black.

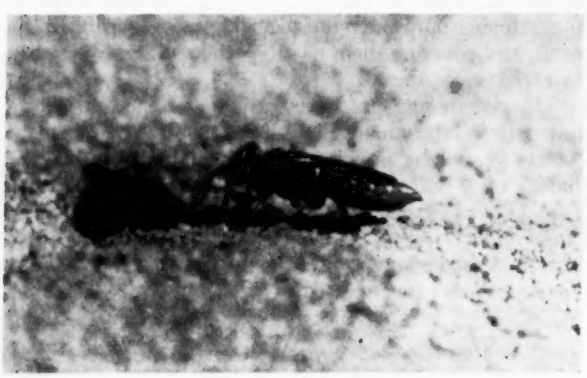

FIG. 1.—Adult *Tachysphex* wasp digging burrow in sandy soil. 2×.

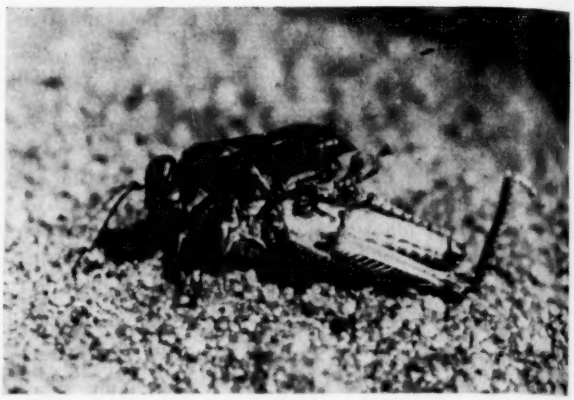

FIG. 2.—*Tachysphex* wasp about to drag paralyzed *Oedaleonotus enigma* nymph to its hole. 2.3×.

Egg.—The eggs are about 2 mm. long and pale gray. They are sausage-shaped and taper slightly to the distad pole. The eggs are placed transversally along the ventral prothorax and mesothorax of the grasshopper nymphs, with the distad pole cemented adjacent to the soft membrane surrounding the procoxa (fig. 3).

Larva.—Full-grown larvae are plump, about 14 mm. long, and without legs. They have a small sclerotized head and well-developed jaws. The body is shiny and chocolate-brown with a bluish cast. While the larva is feeding, its body is frequently curved about the ventral neck of its host (figs. 4 and 5). Within the newly constructed cocoon the mature larva first becomes orange-yellow and later light yellow.

FIG. 3.—Paralyzed 5th instar nymph of *Oedaleonotus enigma* with *Tachysphex* egg placed between prolegs. 8×.

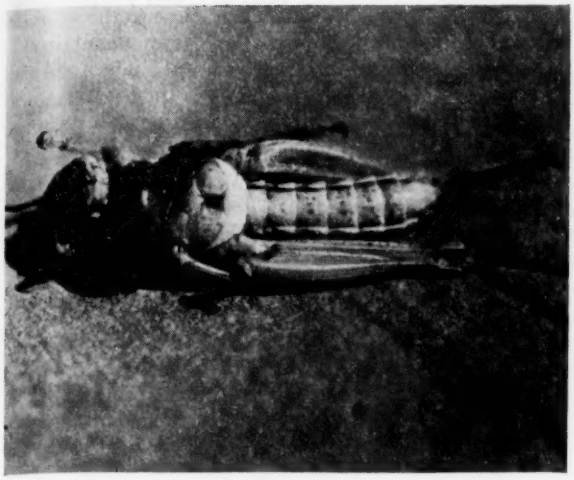

FIG. 4.—Immature *Tachysphex* larva feeding on *Oedaleonotus enigma* nymph. About 3.4×.

Prepupa and Pupa.—In the prepupal stage the head, thorax, and abdomen become differentiated. The prepupae are light yellow, and this color is maintained in the early pupal stage. The pupae have visible free antennae, legs, and wing pads. They darken toward maturity and become deeply colored just before they emerge.

Cocoon.—The cocoons are constructed of sand and soil cemented together by a secretion, and are firm and oval. They are from 10 to 15 mm., with an average of 12 mm. in length and about 6 mm. in width (fig. 6).

LIFE HISTORY AND HABITS.—The adult wasp first digs a hole 1½ to 2 inches deep diagonally into sandy soil or smooth sand (fig. 1). This operation may take from 3 to 8 minutes. The wasp then leaves its open burrow, walks around the entrance several times, and flies off to hunt, sting, and paralyze a nymph of *Oedaleonotus enigma*. The wasp next softens the ventral surface of the host's prothorax and mesothorax by biting the general area in preparation for later oviposition, a process called malaxation, and frequently feeds briefly on the host's body juices as well as on fluids disgorged from its mouth. The wasp then straddles the paralyzed victim and rapidly drags it, frequently by an antenna, directly to the hole (fig. 2), sometimes a distance of 15 yards or more, into which it is pulled. The wasp frequently uses its wings as an aid in moving the host over obstacles, but paralyzed nymphs, even small ones, were never transported by flight alone. Williams (1913), in describing the method used by *T. tarsatus* in transporting a *Melanoplus* nymph to her burrow, says she "seized it by the base of the antennae, and, using her first two pair of legs for running, clasping her prey with the third, proceeded thus at a run, varied with a frequent buzzing hop, to her nest about 20 feet distant. . . ."

FIG. 5.—Nearly mature *Tachysphex* larva feeding on *Oedaleonotus enigma* nymph. About 4×.

FIG. 6.—Cocoons of *Tachysphex* wasps sifted from burrows in sandy soil. 1.3×.

The wasp remains in the hole with her host for 2 to 3 minutes, during which time she lays an egg on the ventral surface of the grasshopper between the prothoracic legs (fig. 3). Williams (1913) observed that *tarsatus* laid "a long, pale greenish, and somewhat curved egg . . . fastened transversally across the prosternum." He further reported (1945) that the egg of *fanuiensis* is 2 mm. long and glued at one end to the cockroach behind the fore coxa and extended obliquely posterad.

After completing oviposition, the female comes outside several times to kick sand into the hole, and then reenters the tunnel to kick and pack the sand in place with her legs. After removing all traces of the entrance, the wasp kicks sand over the general area, then frequently starts digging a second burrow in preparation for another grasshopper victim within a few inches of the first one. This process was repeated by a population of one to five digger wasps per square yard from early morning to late afternoon.

During early June when the grasshoppers were small, the wasps provisioned each burrow with two nymphs, but laid an egg on only one of them. As the grasshoppers become larger, wasps placed only one victim in each hole. At the peak of grasshopper populations the wasps did not always take time to cover the holes, after placing prey

inside; they also left some victims lying paralyzed on the ground. The presence of partly consumed nymphs and living wasp larvae in some of the open holes indicated that wasps occupied them. Others were empty because wasps failed either to find or return to them with prey.

Wasps preyed on grasshoppers at temperatures as low as 46° F. With lower temperatures, or moderate to high winds, adult wasps stopped their work and took shelter in holes they dug in the ground. Williams (1913) reported that "a black species of *Tachysphex*, probably *fusus* or *terminatus*, was observed digging a hole in the sand during the hot afternoon hours, closing its retreat from within." He further stated (1919) that the Fossores usually occupy their nests or other holes at night. Smith (1935) observed that in *Larra analis* Fabricus, a species of a related genus, preying on a mole cricket, the males remained on plants at night while the females were in their burrows. The author did not determine where *Tachysphex* species stayed at night.

The wasps observed in this study preyed almost exclusively on nymphs of *Oedaleonotus enigma*. Only twice did they use nymphs of *Aulocara elliotti* (Thomas) as prey, and once a *Melanoplus* nymph was stung and dragged to a hole. A review of the literature indicates that *Tachysphex* is quite specific in its selection of grasshopper hosts. In the study of other species of *Tachysphex* in Kansas, Williams (1913) found that *fusus* utilized immature *Melanoplus*, *terminatus* Smith used young Acridinae, while *plenoculiformis* Williams used as many as six immature Acridinae per burrow. *T. propinquus* preyed on mature (*Alpha*) *Cordillacris crenulata* (Bruner), *Ageneotettix deorum* (Scudder), and *Trachyrhachis kiowa* (Thomas), and on immature *Opeia* sp., while *tarsatus* used nymphs of *Melanoplus* as well as immature Oedipodinae and Acridinae. Rau (1927) found that *terminatus* used locust nymphs mostly of the species *Chortophaga viridifasciata* (DeG.), and he thought there were several generations of the wasp per year. Williams (1932) believed that in Hawaii *fusus* probably provisioned its burrow with the young of *Oxya chinenis* (Thunberg), the sugarcane grasshopper. Williams (1945) also found that in New Caledonia *Tachysphex fanuiensis* preyed on nymphs and adults of a small cockroach (*Blattella*) *Graptoblotta notulata* (Stal.). Several of the European species of *Tachysphex* are known to prey on immature mantids. Grandi (1930) records *T. costai* Destef., as usually preying on nymphs but occasionally on adult males of *Mantis religiosa* L. and *Ameles abjecta* Cyr.

The wasp always laid its egg after dragging the paralyzed host into the hole, so that oviposition was not observed. The egg hatched in 2 to 3 days. The parasite, with its head within the body cavity of the paralyzed grasshopper and its body usually circling the grasshopper's neck, grew rapidly, killing the host on the second day after hatching. Paralyzed grasshopper nymphs lived 4 and 5 days when the wasp eggs did not hatch. The entire larval period was about 10 days. Molts were difficult to follow, but there were apparently four instars, each lasting a little more than 2 days. Medium-sized larvae sometimes entered the bodies of late-instar nymphs after they had consumed the body fluid, but usually emerged again before they were full grown. Full-grown larvae constructed hard, brittle cocoons in earthen cells close to the sclerotic remains of the consumed hosts. The larvae maintained a curled position within their cocoons until late April and early May of the following year, when they straightened out and entered the prepupal stage. After about a week they entered the pupal stage which lasted about 15 days. The adults emerged when the immature stages of *Oedaleonotus enigma* were present.

A SECONDARY PARASITE.—Small sarcophagid flies identified as *Taxigramma heteroneura* (Meig.)[4] were numerous over the area where the studies of *Tachysphex* sp. were made. These flies, about 3 to 4 mm. long, showed an interest in the activity of the wasps, and in the paralyzed grasshopper nymphs as they were dragged into the burrows. The flies were seen to enter the holes soon after the wasps dug them. The author did not determine when *heteroneura* infested the grasshopper, but it was probably after the wasp had stung and paralyzed the nymph but before it covered its burrow. Williams (1913), in his work on the Larridae, observed and described the habits of a similar small dipteran which he did not have identified. He stated that this fly deposited its own young on the food intended for the larrid grub, and he thought that the maggot destroyed the wasp egg before feeding on the paralyzed host. Newcomer (1930) studied two small sarcophagid flies *Hilarella hilarella* (Zett.) and *Metopia leucocephala* (Rossi) as well as *heteroneura* which he found preying upon the digger wasp *Podalonia luctuosa* (Smith), a predator of cutworms. He gives an interesting account of the life history and habits of these flies. Of *Hilarella hilarella* (Zett.), a species with habits apparently similar to those of *heteroneura*, he says that living maggots are sticky, and are placed on the prey by the fly as it is dragged into the hole or are dropped into the hole after the prey has been dragged in. He further says, "I have observed these maggots feeding on the egg (of the wasp) in several instances although they do not always seem to do this, as in one or two cases cutworms were dug up that had both maggots and a wasp feeding on them."

Nymphs paralyzed and buried by *Tachysphex* spp. had wasp larvae feeding on them 3 days later, and on the fifth day the same nymphs were infested with several small dipterous larvae, which started pupating on the seventh day. The number of maggots ranged from 2 to 12 per provisioned hole, and the maggots consumed both the grasshopper nymphs and the wasp grubs. Extensive digging and sifting of the soil showed that about 10% of the grasshopper nymphs and wasp grubs were so affected. Dipterous puparia were brown and 4 to 4.5 mm. long. Adult flies emerged from some of them in about 8 to 10 days, indicating that several generations of this fly occur during the season. From other puparia held at 64° F. in the laboratory flies emerged about 2 weeks after they were removed to room temperature the following April.

SUMMARY.—Three species of digger wasps of the genus *Tachysphex* were observed preying on the grasshopper *Oedaleonotus enigma* (Scudder) during an outbreak in

[4] Determination made by C. W. Sabrosky, Entomology Research Branch, U. S. Dept. of Agriculture.

south-central Idaho in 1954 and reduced populations of 20 to 30 per square yard to 1 in 5 square yards over the area under study during June.

The adult wasp dug shallow holes in the soil, then hunted, stung, and paralyzed grasshopper nymphs and dragged them into the burrows. An egg was laid on each host and the entrance of the burrow covered. The egg hatched in 2 to 3 days. The larva killed its host on the second day after hatching, and completed its growth in 10 days. The species overwintered as a mature larva within a hard soil-covered cocoon. Pupation took place early in May, and emergence occurred from mid-May to mid-June.

About 10% of the wasp larvae and paralyzed nymphs were attacked by a small sarcophagid fly, *Taxigramma heteroneura* (Meig.), which consumed both the *Tachysphex* larva and its host.

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The Emergence of Flies Reared from Grass Silage in Michigan[1]

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Increased numbers of flies were evident during an investigation of fly control in Michigan State University dairy barns (King *et al.* 1953, Guyer *et al.* 1954). The house fly, *Musca domestica* L., and the stable fly, *Stomoxys calcitrans* (L.), were the principal species responsible for these heavy populations. When an investigation was undertaken to ascertain the larval habitats of these species it was discovered that grass silage adjacent to the dairy barns was serving as a larval medium for several species of flies. Many crops and materials have been utilized in an effort to provide better preserved roughage (Bender 1937). Certain structural adaptations in the storage of silage such as the open bunker type and the self-feeding upright silos have increased the exposed surface area for larval development. The adoption of the self-feeding of hay and silage in loose-housing structures (Cargill *et al.* 1955) has been responsible for the build-up of concentrated fly populations.

The objectives of this investigation were to determine the species composition and to study the ecological requirements of the insect populations associated with grass silage. Series of samples of maggot-infested silage were collected in 1954 and 1955 at the storage sites and transferred to rearing jars. Daily observations were made of the emerging adults and the specimens were removed and preserved for taxonomic study. In addition to the periodic sampling at each site, samples of a quantitative nature were taken at sites VI and VII during the 1955 investigation. The quantitative samples consisted of 2-quart samples of silage taken from the top 6 inches, the middle of the pile at the exposed surface and from the drainage area.

The seven sites sampled are outlined in table 1. The type of sample taken at the various sites was contingent on the silo construction and the method of manipulation of the silage. An effort was made to sample as many as possible of the areas where grass silage might be available for egg deposition and larval development. Seven crops were utilized in the formulation of the silage mixtures.

DISCUSSION OF RESULTS.—Table 2 indicates that 20 species of flies were identified from the insects emerging from the silage samples. There were 10 families of Diptera represented by the 20 species. Two species, *Musca domestica* and *Chrysomyza demandata* (F.) made up 75% of the flies emerging from the silage. *M. domestica* was the most widely distributed species, being reared from all of the collections where maggots were present.

The complex interaction of ecological factors made each storage site a specific biological habitat. Within each silage storage area smaller biological communities developed as a result of the variations in microclimate which occurred in and near the silage. The variation in moisture content of the silage appeared to be the most important single factor which affected the maggot fauna. This was especially evident at site V where the silage was

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Table 1.—Data on silage composition and storage.

Site No.	Location and Year	Method of Sampling	Silo Construction	Date Silage Was Put Up	Type of Silage	Preservative
I	Redman barn (1954)	Random at all depths	Trench silo, cement sides and base	July 1953	Oat 50% and pea 50%	none
II	Exptl. barn (1954)	Random at base of silage and from drainage	Open stack	June 1954	Brome 75% and alfalfa 25%	none
III	Pen barn (1954)	Random from feeding area at bottom of silo	Self-feeding upright silo	June 1954	Brome 75%, alfalfa and small amount of clover	none
IV	ABA barn (1954)	Random from around self-loading entrance	Glass-lined Smith Harvestor upright	June 1954	Brome 90% and alfalfa 10%	Molasses
V	Biebesheimer barn (1954)	Random from open end of silage	Trench silo, treated wooden sides with cement base	June 1954	Timothy and brome	none
VI	Redman barn (1955)	At definite areas in silage and drainage	Trench silo, cement sides and base	May 1955	Alfalfa 60% and rye 40%	none
VII	Exptl. barn (1955)	At definite areas in silage and drainage	Trench silo, treated wooden sides with soil base	May 1955	Lower portion Balbo rye-top alfalfa brome	none
VIII	Exptl. barn (1955)	Random from debris around silo	Upright silo	May 1955	Alfalfa 60%-Balbo rye 40%, small amount of clover	none
IX	Exptl. barn (1955)	Random from inside silo—as feeding	Upright silo	May 1955	Alfalfa 60%, Balbo rye 40%, small amount of clover	none
X	Pen barn (1955)	At face of feeding area	Chopped hay (dry)	June 1954	Alfalfa-brome	none
XI	ABA barn (1955)	Random from drainage area	Glass-lined Smith Harvestor upright	June 1955	Brome 90%-alfalfa 10%	Sodium bisulfite

extremely dry and a maggot population did not develop. Although several different crops were used in varying formulations in preparing the silage it was not possible to associate any specific species or complex of species with a definite herbage mixture of the silage.

Generally maggot populations developed under the following three conditions:

(1) In the drainage areas where the excess moisture from the silage collected.

(2) In the outer 8 inches of exposed silage when sufficient moisture was present.

(3) In the feeding areas where silage became mixed with manure and debris and provided a seemingly ideal medium for larval development.

An investigation was conducted to ascertain what conditions favored or inhibited the development of the various species. At sampling sites VI and VII, quantitative samples were taken from the top and middle of the exposed silage as well as from the drainage areas (Table 3). As in the discussion of total fly emergence, *M. domestica* and *C. demandata* were the most numerous species reared during the quantitative study at sites VI and VII. At site VI the seepage from the silage drained onto the cement apron of the bunker as well as on the soil. The collection made from the drainage area differed in its species composition from the top and middle samples. In the wet, putrid drainage *M. domestica* was the only species present with the exception of four specimens of *Leptocera exiguella* Spuler. *C. demandata* was the predominant species reared from the samples taken from the top of the silage. Often when visits were made to sites VI and VII large concentrations of *C. demandata* adults were evident on or near the top of the silage piles. The other species of flies were distributed generally among all three sampling locations at sites VI and VII. It is impossible to associate distinct ecological requirements with most of these flies due to their wide distribution.

At site VII where quantitative samples were taken, the differences in sampling areas within the silage were not as distinctly delineated as at site VI. The drainage area at site VII did not consist of the drainage alone but also contained decaying silage. The insect population at site VII did not have the distinct species composition evident in the samples from the three different areas at site VI.

It was impossible to ascertain specifically what ecological conditions favored the egg laying and development of the stable fly, *Stomoxys calcitrans*. During 1954 at site I a concentrated population of stable flies was present in oat-pea silage. The stable fly larvae were especially numerous in the decaying silage along the sides and at the bottom of the exposed surface where cattle were feeding. The 1955 samples, taken at site VIII, which consisted of a mixture of alfalfa-Balbo rye mixed with debris and allowed to accumulate around the unloading area of the silo were predominantly populated with stable flies. At many of the other sampling sites these same conditions which appeared to attract stable flies were also evident but a stable fly population did not develop.

Although a specific study of the seasonal variation in the maggot population was not made, it was apparent that differences did exist. Maggots were most numerous during the last 2 weeks of July. Maggots were plentiful in the silage during August and September but did not increase to the high numbers present during July. At several of the silage sites maggots were active in the silage until it became frozen in November.

Three species of *Drosophila* were responsible for 75% of the population from the samples taken at site IV. The silage at site IV was an alfalfa-brome grass combination with molasses added as a preservative. All of the *Drosophila* specimens with the exception of a single representative from the site III collection were taken from silage at site IV which contained the molasses preservative.

Table 2.—Dipterous insects reared from grass silage.

Family and Species	Numbers of Emerging Insects Site Number											Total Emergence of Each Sp.
	1954					1955						
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
MUSCIDAE												
Musca domestica L.	4		20	19		198	27	1	118	105	309	801
Stomoxys calcitrans (L.)	33		6					24	1	1	1	66
Ophyra leucostoma (Wied.)		3	1					1				5
OTITIDAE												
Chrysomyza demandata (F.)	5					482	70				2	559
SCATOPSIDAE												
Scatopse fuscipes Meig.	4	1	8			33		14				60
SEPSIDAE												
Themira putris (L.)		15	4									19
SYRPHIDAE												
Syritta pipiens (L.)			1			112						113
Tubifera tenax (L.)			6									6
DROSOPHILIDAE												
Drosophila busckii Coq.			1	18								19
D. melanogaster Meig.				1								1
D. hydei Sturt.				17								17
CALLIPHORIDAE												
Pollenia rudis (F.)			1									1
LYCORIIDAE												
Bradysia sp.		1										1
SPHAEROCERIDAE												
Leptocera limosa (Fall.)			5									5
L. ferruginata (Stenh.)			2				2		5			9
L. exiguella Spuler	2	1	9			4	2		1			19
L. elegans Spuler	5	10	21			4			45			85
L. mirabilis (Collin)	1											1
L. acutangula (Zett.)							1					1
MILICHIIDAE												
Desmometopa sordida (Fall.)						22						22

Table 3.—Insect distribution in the silage.

Family and Species	Sampling Site VI			Sampling Site VII			Total Emergence of Each Sp.	Per Cent of Total
	Top	Middle	Drainage	Top	Middle	Drainage		
MUSCIDAE								
Musca domestica	24	5	169		20	7	225	23
OTITIDAE								
Chrysomyza demandata	467	15		39	2	28	551	57
SCATOPSIDAE								
Scotopse fuscipes	16	15					31	3
SYRPHIDAE								
Syritta pipiens	99	13					112	11
SPHAEROCERIDAE								
Leptocera ferruginata					2	24	26	3
L. exiguella			4		1	1	6	[a]
L. acutangula						1	1	[a]
MILICHIIDAE								
Desmometopa sordida		18					18	2

[a] Made up less than 1%.

SUMMARY AND CONCLUSIONS.—The increased interest in the storing of grass silage has extended the problems associated with fly control. The handling of silage and the self-feeding of hay in and near loose-housing structures has also provided conditions favorable for supporting concentrated fly populations. The silage formulations which have been used have provided ideal habitats for the development of the immature stages of several species of flies.

The species composition and numerical distribution of the larval populations in silage appear to be dependent on several ecological factors. The moisture content of the silage appears to be the most important single factor affecting the extent of the fly population. Other factors which are important in determining the magnitude of the fly population are the type of construction of the silage storage structure, the type of preservative added, the area of exposure of the silage and the method of feeding.

There is a wide variation in the species composition of flies reared from grass silage. Twenty species of flies belonging to 10 families were identified from the silage samples. Two species, *Musca domestica* and *Chrysomyza demandata*, made up 75% of the flies emerging from the silage. Information from this study indicates that the species composition may differ at various locations in and near the silage. There was a seasonal variation in the total maggot population and the species composition of this population. The inability to provide satisfactory fly control in buildings closely associated with the storage of silage may frequently be explained by the concentrated populations present in the silage adjacent to dairy barns.

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Residual House Fly Control in Dairy and Beef Barns in South Carolina[1]

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Although control of house flies with poison baits has been effective in many states, there still exists a need for efficient and low-cost residual sprays to control both house flies and stable flies. Dahm & Raun (1955) working in Iowa, found residual sprays very useful in areas where sprayers were available and where approximately one-half of the flies on the farm were stable flies which were not normally attracted to fly baits. Organic phosphates such as malathion, Diazinon, Am. Cyanamid 4124, Chlorthion, and Bayer 21/199 have shown promise as residual insecticides for house fly control (Wingo 1954, Hoffman 1954, Hansens *et al.* 1955, and Hansens & Scott 1955). The experiments, results of which are reported herein, were designed to evaluate various organic phosphate insecticides in dairy and beef barns under conditions existing in South Carolina, and to develop practical but inexpensive methods of applying residual insecticides.

MATERIALS AND PROCEDURES.—The insecticides used in these tests were 25% Chlorthion wettable powder, 57% malathion emulsion, 25% Diazinon wettable powder, 50% Am. Cyanamid 4124 wettable powder, 25% Am. Cyanamid 4124 emulsion, 25% Bayer 21/199 wettable powder, and 55% malrin emulsion[4] (containing 18% malathion and 35% Perthane). The concentrations used are shown in table 1. The only attractant added to any of these materials was molasses which was added only to malathion.

The barns were checked at weekly intervals after application, employing the spot counting method for evaluating the degree of control obtained. This method is an adaptation of the method used by Pimentel (1950) and Goodwin (1953) in New York dairies. The average South Carolina dairy barn is small with 6 to 20 stanchions and is used for milking only. This method of counting flies involves counting all the house flies on six 2-square-foot areas where the flies normally rest. These areas are the sunny entrance, sunny window, stanchions, gutter, manger, and the feed room floor. The following categories were established to evaluate the degree of control in the barns: (1) 0–15 flies = excellent, (2) 15–50 flies = satisfactory, and (3) more than 50 flies = unsatisfactory. Ratings of excellent and satisfactory were considered as being practical house fly control conditions. This same method was used in beef barns except the flies on the feed rack were counted instead of those on the stanchions.

METHODS OF APPLICATION AND RESULTS.—During 1954 only the overall method was used in applying residual insecticides, while in 1955 the overall and spot methods were employed.

Overall Method.—This method is the standard one used

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[4] A product of Agricultural Specialties, Dallas, Texas.

Table 1.—Weeks of excellent and satisfactory residual control of house flies in South Carolina dairy and beef barns during 1954 and 1955. Clemson, S. C.

METHOD	INSECTICIDE[a]	AMOUNT PER 100 GALLONS	NUMBER OF BARNS	WEEKS CONTROL	
				Range	Average
	Dairy Barns, 1954				
Overall	25% Chlorthion WP	8 lbs.	4	2–3	2.5
	25% Chlorthion WP	32 lbs.	3	7–8	7.3
	57% malathion EC[b]	2 gals.	6	3–4	3.3
	25% Diazinon WP	8 lbs.	4	2–4	2.7
	25% Diazinon WP	32 lbs.	3	6–7	6.3
	50% Am. Cyanamid 4124 WP	16 lbs.	2	6–7	6.5
	25% Am. Cyanamid 4124 EC	4 gals.	4	5–6	5.7
	Dairy Barns, 1955				
Overall	Chlorthion	32 lbs.	2	2–4	3.0
	25% Diazinon WP	16 lbs.	3	4–8	6.0
	25% Diazinon WP	16 lbs.	6	—	4.0[c]
	50% Am. Cyanamid 4124 WP	16 lbs.	6	3–7	5.0
	55% malrin EC	2 gals.	3	0–10	4.0
	25% Bayer 21/199 WP	16 lbs.	6	3–6	4.0
Hose Sprayer					
	55% malrin EC	2 gals.	3	0–6	4.0
	25% Am. Cyanamid 4124 EC	4 gals.	2	4–6	5.0
Spot					
	25% Diazinon WP	32 lbs.	2	3–7	5.0
	50% Am. Cyanamid 4124 WP	32 lbs.	3	3–8	5.0
	Beef Cattle Barns, 1955				
Overall	25% Diazinon WP	16 lbs.	2	—	2.0
	50% Am. Cyanamid 4124 WP	16 lbs.	3	1–5	3.0
	25% Chlorthion WP	32 lbs.	1	—	4.0
	25% Bayer 21/199 WP	16 lbs.	1	—	2.0

[a] WP refers to wettable powders and EC to emulsifiable concentrates.
[b] Two gallons of molasses was added to this insecticide.
[c] Material still effective when fly counts discontinued.

in applying residual insecticides, and involves spraying the entire inside surface of the barn except the floor. A hydraulic sprayer was used to apply the insecticides, which were applied at 300 p.s.i. at the rate of 1 gallon per 250 to 275 square feet.

In 1954 a total of 28 dairy barns were treated using this method. The materials used, concentrations, and the results obtained are given in table 1. Three materials, Am. Cyanamid 4124, Chlorthion, and Diazinon gave over 4 weeks control. The house fly population was low during this experiment due to extremely dry weather, which may have resulted in longer residual control. This experiment was repeated during 1955 when a total of 26 dairy barns and 7 beef barns were sprayed using the materials and concentrations shown in table 1. In general, the periods of satisfactory control were not as long in 1955 which was a more normal year in relation to weather conditions and fly breeding. The results shown in table 1 reveal that Diazinon, Am. Cyanamid 4124, malrin and Bayer 21/199 gave satisfactory control of house flies for 4 weeks or longer.

A device designed so that it can be attached to a garden hose has been used for spraying plants for several years. One device, Ortho Livestock Sprayer,[5] has been used by several dairymen as a means of applying fly sprays to the barn as well as on the animals. This sprayer is designed for liquid insecticides only, and graduated to apply from 1 to 10 gallons of finished spray. This device was included in the experiment to compare its effectiveness versus the hydraulic sprayer as equipment for applying residual insecticides to dairy barns.

A total of five barns was sprayed with this device using malrin and Am. Cyanamid 4124 emulsifiable concentrates as overall sprays. The degree of control was comparable to that obtained with these materials when applied with hydraulic sprayer. This device is inexpensive, easy to use, and practical for small dairymen. The two disadvantages are: (1) In order to obtain the desired concentrations in the finished spray only emulsifiable concentrates can be used, and (2) there is a possibility of contaminating the water system with insecticides.

Spot Method.—The spot method (Pimentel 1951, Goodwin 1953) involves spraying the areas where house flies congregate most frequently. These areas were around the doors, the windows, and the ceiling above the center alley ways. In order to reduce the hazards of milk contamination the areas above the mangers and drinking fountains were not sprayed. The concentrations of insecticides were doubled but the gallonage applied was reduced by one-half. The same type of hydraulic equipment was used in the spot method as used in the overall method.

A total of five barns was sprayed in 1955 using Diazinon and Am. Cyanamid 4124. The results obtained compare very favorably with those obtained with the two materials when applied using the overall method.

DISCUSSION AND CONCLUSIONS.—The authors do not consider an insecticide practical as a residual spray unless it gives at least 4 weeks of satisfactory control. Since 1954 was unfavorable for house fly breeding due to dry weather, the results obtained in 1955 will more nearly represent the true picture of the longevity of these organic insecticides when applied as residual sprays under South Carolina conditions. Diazinon, Am. Cyanamid 4124, malrin, and Bayer 21/199 all gave satisfactory control for an average of 4 weeks or more. Malrin exhibited extreme limits in the degree of control obtained in dairy barns. This range was from 0 to 10 weeks of satisfactory control. Malrin gave house fly control for less than 1 week in two of the experimental barns. Both of these barns were similar to others in this experiment with respect to sanitation and house fly population.

The spot method of applying residual insecticides gave control in dairy barns approximately as long as the overall method when Diazinon and Am. Cyanamid 4124 were used. This spot method reduces hazards of milk contamination and requires only one-half the gallonage to treat a barn that is required in the overall method.

When various organic phosphate insecticides were applied in beef barns, where the fly breeding potential was extremely high, the period of control was reduced considerably. Diazinon gave control only 2 weeks in beef barns compared to 6 weeks in dairy barns. This demonstrates the advantage of removing the majority of the larval habitats to prevent fly breeding. The dairy barns were cleaned and hosed down daily, the beef barns were cleaned only several times per year. The control of flies in these barns is extremely difficult unless efforts are made to reduce the larval habitat.

SUMMARY.—A total of 64 dairy barns and 7 beef barns

[5] California Spray Chemical Company, Richmond, California.

were sprayed in South Carolina during 1954 and 1955 for residual control of house flies. The two methods of application employed were the overall spraying where a hydraulic sprayer and a garden hose sprayer were used, and the spot spraying method.

The house fly populations were low during 1954 because of extremely dry weather, but during 1955 the weather conditions and house fly breeding were normal. Diazinon, Chlorthion, Am. Cyanamid 4124, malrin, and Bayer 21/199 gave satisfactory control for 4 weeks or longer.

When beef barns with high fly breeding potential were treated the period of control was reduced approximately one-half that obtained in dairy barns with average sanitation. Clorthion gave the best control in the beef barns.

The garden hose sprayer proved to be a practical and inexpensive method of applying emulsifiable concentrates, and the same residual control was obtained as when the hydraulic sprayer was used to apply the same insecticides.

The spot method was equal to the overall method in the residual control obtained with two insecticides.

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Air Drift of Spider Mites[1]

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During the past five years (1950–1955) the authors have obtained considerable information on wind-drift patterns of the following mites: the citrus red mite, *Metatetranychus citri* (McG.); the avocado brown mite, *Oligonychus punicae* (Hirst); the six-spotted mite, *Eotetranychus sexmaculatus* (Riley); and the two-spotted spider mite, *Tetranychus telarius* (L.). This information was obtained as a part of studies on the biological control of plant-feeding mites in southern California.

It has been known for some time that certain spider mites commonly drift on air currents. In 1913, Stabler reported that E. E. Munger of Yuba City, California, had demonstrated wind drift of red spiders from an almond grove. Mr. Munger placed sheets of sticky fly paper on boards various distances and heights down wind from an infested almond grove. The most distant trap was placed 50 feet from the ground, on top of a school house, 650 feet from the orchard. The next day many red spiders were found on the sticky paper.

Through the use of a special trap attached to airplane wings, Coad (1931) studied airborne insect populations at altitudes of from 50 to 14,000 feet. By this method he collected mites (undetermined species) at 10,000 feet elevation.

Ebeling (1934) observed citrus red mites lowering themselves from one leaf to another by means of silken threads. Further observations showed that this occurred only when the mite population on the leaf had become large and the chlorophyll was nearly depleted. His conclusion was that if mites were to lower themselves in this manner in the open, especially on large trees, it would be possible for the wind to blow them away and carry them long distances.

Gilliatt (1935) concluded from orchard studies that there was no large-scale wind drift of the European red mite, *Metatetranychus ulmi* (Koch). He stated that it seems possible that more mites are transported by light or moderate winds than by gales.

From field experiments in apple orchards in Washington, Newell (1941) concluded that wind drift of the Pacific spider mite, *Tetranychus pacificus* McG., is important largely because the mite may thus reinfest orchards. He also concluded that more mites are transported by light winds than by gales.

Marlé (1951) investigated field and laboratory conditions under which there was air dispersal of the European red mite, *Metatetranychus ulmi* (Koch). Her findings indicated that the great majority of those migrating were females, which dispersed at periods of peak population in each generation; the numbers moving were dependent upon the degree of infestation of the host. Parachute threads were normally formed in still air following relatively calm periods, when temperatures were above 70° F. and relative humidities were low.

CITRUS RED MITES.—*In the Orchard.*—On August 23, 1950, detailed field studies on the citrus red mite were

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started in the Basinger Valencia orange grove at Riverside, California. This grove had received an application of oil spray on August 17 which practically eliminated the citrus red mites. The grove was therefore examined at frequent intervals to determine when and under what conditions the first measurable mite population would appear.

No mites were seen in the grove until January 18, 1951, 5 months after treatment. A tree-to-tree search on this date revealed that within a period of about 5 minutes several female mites could be found on the trees near a road bordering the grove. (No males or intermediate stages could be found.) The mites were more difficult to find on trees more distant from the road. During a careful search of one tree near the road, first one female mite and then several more females were observed to alight on the leaves. They were apparently drifting into the experimental grove from a grove across the road.

Examination of the neighboring grove across the road disclosed a high population of citrus red mites. The mites were spinning down from the leaves of the infested trees by the thousands, each mite being suspended by a single silken thread. A gentle breeze then picked them up and carried them off in the direction of the experimental grove.

This mass wind-borne migration of mites continued for several days. By January 21, three days after the first mites were observed to be drifting into the grove, the female mite population on trees three rows from the road averaged about four mites to each 32-leaf sample. By this time eggs could be found on leaves infested with mites. As there were practically no natural enemies in the experimental grove, the mite population increased steadily. By March 25, the female mite count on trees in the third row from the road averaged 420 per 32-leaf sample. Thus a serious infestation developed as a result of mites drifting in from a neighboring grove.

In the Greenhouse.—The relation of air-drift activity of citrus red mites to their population densities on greenhouse-grown lemon trees is shown in figure 1. These data were obtained from an experiment in an insect-proof greenhouse (12×12 feet) between January 27 and May 19, 1955. The mites were feeding on six 2-year-old lemon trees. There were no natural enemies of mites in the greenhouse. Automatic humidifiers ensured that the relative humidity would not drop below 50%. The temperature of about 80° F. was maintained by means of forced air, which entered the greenhouse through ducts opening below a duckboard floor. Thus gentle air currents issued upwards from the entire floor area.

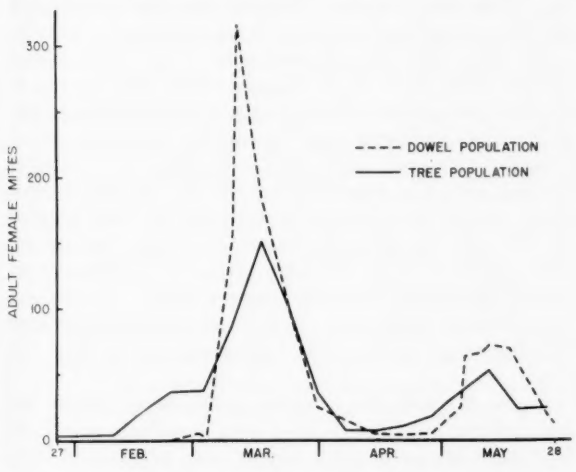

FIG. 1.—Relation between the average numbers of female citrus red mites per 10-leaf samples from six young lemon trees in a greenhouse ("tree population") and the average numbers of mites carried by air drift to four 7½-foot dowels, one in each of the four corners of the greenhouse, during 24-hour periods ("dowel population"), January 27 to May 28.

Figure 1 shows the population trends of mites on the lemon trees from January 27 to May 19. Each point on the "tree-population" curve represents the average number of females for each 10-leaf sample on the date indicated. This average was two per sample when the experiment started on January 27. During the next 50 days the mites steadily increased in numbers to reach a peak of 155 per sample on March 17. After this date they declined sharply to reach a low of eight per sample on April 14. This low was followed by a second peak of 52 per sample on May 12 and a decline to 23 per sample by May 19.

The "dowel-population curve" shown in figure 1 represents the number of mites carried by air drift from the citrus trees to four wooden dowels, one in each of the four corners of the greenhouse, within 24-hour periods. These dowels were wooden poles ⅜ inch in diameter and 7½ feet long, each one extending from the floor to within a few inches of the ceiling, and each one placed about 2 feet from the walls of its respective corner. The bases of the dowels were ringed with "Deadline" so that no mites could crawl onto the dowels from the floor. Since the dowels stood perpendicular to the floor, there was little chance for the mites to spin free and drift away from them. Therefore it is thought that the number of mites removed from the dowels at the end of each 24-hour period represent fairly accurately the number which had drifted to them within that period. Only females were found on the dowels.

Figure 1 shows that there was very little wind drift of mites from the trees until March 10, when the average population was 86 for each 10-leaf sample. On this date 154 mites were collected from the four dowels. The peak of air-drift activity (315 mites) was reached 1 day later, March 11. After this peak the drift activity of the mites declined sharply in correlation with the mite population on the trees. The same general correlation may be noted at the time of the second peak and decline of mites on the trees in May.

The data in figure 1 show that the mite populations on the trees attained higher numbers during the March peak, before marked air-drift activity, than they did during the May peak. This very likely reflects a difference in the quantity and/or quality of food available to the mites at these times. It was observed that in each instance the maximum rate of increase in the mite populations occurred shortly after periods of flush growth. The subsequent rapid decrease in mite populations presumably resulted largely from mites' spinning off the leaves and away from the trees after the food supply had been depleted.

Numerous other field and laboratory experiments have

shown that the citrus red mite population density at which air-drift activity reaches its peak may vary greatly from tree to tree and from grove to grove. For example, the mites began to spin off the leaves of one lot of greenhouse-grown citrus trees before the population was two females per leaf. The mite population on this particular group of plants never exceeded two mites per leaf. A lemon tree in a grove under close study, however, developed a population of approximately 100 female citrus red mites per leaf before spinning and air-drift activity became intense.

AVOCADO BROWN MITES IN THE ORCHARD.—Detailed studies of the natural balance of the avocado brown mite in the Palmer avocado grove at Carlsbad, Calif., covered a period of 85 days, from July 15, through October 8, 1954. During this entire period the mite populations on certain trees were under constant observation during daylight hours. On several occasions observations were continuous for periods of 24 hours.

This grove is about ½ mile from the ocean. Air temperature extremes during the entire study period ranged from a high of 86° F. to a low of 46° F. The highest temperature for a 24-hour period was generally reached between 12 noon and 1:00 P.M.; the lowest, between 4:00 and 5:00 A.M. The relative humidity at midday generally ranged from 50 to 80%. At sunset, however, the relative humidity (accompanied by a sharp drop in temperature) would rapidly rise to 100% and remain there through the night until about 7 or 8 o'clock or, at times, until 10 o'clock the next morning. During the day there were prevailing onshore breezes which generally died down by sunset.

On evenings when the air was calm, the female avocado brown mites began spinning off the leaves soon after sunset. Except when weather conditions were adverse, spinning and drifting activities continued throughout the night, and since the mites and their webs reflected light rays from a flashlight beam, their movements were easily followed. Mites were observed to be suspended on threads up to 4 feet in length. When there was insufficient air movement to carry them away, they were frequently seen to lower themselves on their silken threads until they came in contact with a lower leaf or with the ground. Commonly, threads of a number of mites became so entangled that all appeared to be suspended on a single thread. As many as 38 mites were observed in such a group. When the supporting strand was broken by an air current, the entire group of mites drifted away as a unit.

Almost imperceptible air currents within and around the trees were sufficient to break the threads and carry the mites, singly or in groups, from tree to tree. Mites were also commonly observed drifting upward on rising air currents until they were lost in the darkness.

In order to gain some information on this drifting of mites at higher elevations, a 20-foot, 2×2-inch pole was erected in the grove about 20 feet from the nearest of four surrounding trees. This pole was covered on three sides with white aluminum strips. "Deadline" barriers were placed along the edges of, and at 1-foot intervals across, the aluminum strips. (The fourth side of the pole was left free of "Deadline" for convenience in handling.) Thus mites drifting onto the pole were trapped in a restricted area having a clean white surface to facilitate counting. During a single 12-hour overnight period when the mite population in the grove was at its height, a total of 54 brown mites was collected from the top 2 feet of the pole (an area of 1 square foot). This upward drift of such large numbers of females undoubtedly results in distributing mites to neighboring groves and possibly over distances of miles in a short period of time.

In the course of these experiments it was learned that the avocado brown mites do not spin off leaves to migrate when the wind is blowing. Thus it is not necessarily true that the greatest airborne migration of these mites is in the direction of the prevailing wind. For example, of the three mite-collecting sides of the 20-foot pole previously described, one faced the prevailing wind, one faced away from it, and one faced at right angles to the prevailing wind. During one 12-hour overnight period when a total of 54 brown mites was collected from the top 2 feet of the pole, 26 of the mites were taken from the windward side, 24 from the leeward side, and 4 from the side facing at right angles to the prevailing wind.

From the data obtained from the experiments in this grove, and from data obtained from other experimental avocado groves, it is now known that at times the greatest airborne movement of avocado brown mites may actually be in the direction opposite that of the prevailing winds. This being true especially in areas where air drift at night is opposite the direction of the prevailing diurnal breeze.

A close study was made of the spinning and wind-drift activities of avocado brown mites on a tree in the Palmer grove which had been sprayed with DDT. The spray having largely eliminated their natural enemies, the mites developed in exceptionally large numbers. Thus the spinning and wind-drift activities of the mites were somewhat greater on this tree than on surrounding trees.

In order to estimate the number of mites spinning or drifting onto leaves of the DDT tree, five twig terminals bearing 5 to 10 leaves each were isolated from the tree by a "Deadline" barrier. The only way that mites could get to these isolated leaves was by spinning down or drifting to them from other leaves on the tree, or by drifting in from other trees. From August 28 through October 8, all mites were removed from each of the isolated terminals at 10 o'clock each morning. The number of mites removed from the terminals daily gave some idea of the number drifting onto the terminals within the 24-hour period. This was not an accurate measure of the total number of mites drifting onto the terminals within this period, however, for many of the mites were observed to spin off the terminals after having touched them. The drifting activity of the mites was therefore somewhat greater than that indicated by the figures obtained during these studies.

Figure 2 shows the average numbers of females per leaf drifting onto the isolated twig terminals within 24-hour periods, compared to the average numbers of mites per leaf obtained from biweekly samples of the rest of the tree.

In general, the spinning and drifting activities of the brown mites on the isolated terminals (fig. 2) increased in intensity from two mites per leaf on August 28 to a sharp peak of slightly over 20 mites per leaf on September 11. Spinning and drifting activities were considerable,

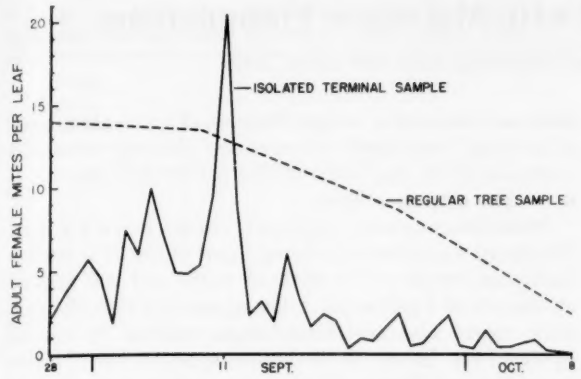

FIG. 2.—Relation between the average numbers of female avocado brown mites per leaf drifting onto isolated twig terminals of an avocado tree during 24-hour periods ("isolated terminal samples") and the average numbers of female mites per leaf in biweekly samples ("regular tree samples") from the same tree, August 28 to October 8.

however, until the experiment was terminated on October 8.

The avocado brown mite population on the DDT-sprayed tree (fig. 2) declined from an average of 14 females per leaf on August 8 to about 2.5 females per leaf when the experiment was discontinued on October 8. As all species of natural enemies had been killed by the DDT deposit on the tree, this decline was doubtless due to a change in leaf condition which both lowered the reproductive rate of the mites and caused them to migrate.

During the experimental period frequently between $\frac{1}{4}$ and $\frac{1}{2}$ of the entire adult female mite population moved from tree to tree or from leaf to leaf every 24 hours. It should be pointed out, however, that during this period the number of females per leaf represented only about 15% of the total mite population. Therefore, newly matured females were constantly being produced to replace a portion of those migrating from the leaves. It should also be mentioned that the leaves on the isolated terminals did not suffer severe feeding damage as did most of the leaves on the rest of the tree. For this reason the mites that migrated to the leaves on the isolated terminals doubtless found them more suitable and had less tendency to leave. This probably accounts for the fact that on September 11 the average number of females per leaf on the isolated terminals was much greater than the average number of mites per leaf on the rest of the tree.

SIX-SPOTTED MITES IN THE ORCHARD.—Studies were also made on the spinning and drifting activities of the six-spotted mite in the Palmer avocado grove. Populations of both avocado brown mites and six-spotted mites occurred on the trees during these experiments. The avocado brown mites fed mainly on the upper surface of the leaves, and the six-spotted mites on the under surface of the leaves. Although the six-spotted mites were fewer in number, they caused much more serious damage to the trees than the avocado brown mites. The spinning and drifting activities of the six-spotted mites were similar in every respect to those of the avocado brown mites.

TWO-SPOTTED SPIDER MITES IN THE GREENHOUSE.—A number of infestations of the two-spotted spider mite on citrus trees in a greenhouse were studied. In no instance was this mite observed to spin from infested trees to neighboring uninfested trees.

SUMMARY.—The air drift of four species of mites was studied. In a mass, wind-borne migration of the citrus red mite, *Metatetranychus citri*, female mites were carried by gentle breezes from a heavily infested citrus grove, across a road, to an uninfested grove. A mite population of economic importance soon developed in the previously uninfested grove.

Female citrus red mites in a small greenhouse were carried by air currents from six lemon trees to four $\frac{3}{8}$-inch wooden dowels, one in each corner of the greenhouse, about 2 feet from the walls and extending from the floor to within a few inches of the ceiling. The number of mites drifting to the dowels daily was correlated with the mite populations on the trees. There was little wind drift of mites from the trees until the population was about 86 females per 10 leaves. A rapid increase in numbers of mites followed periods of flush growth. The spinning-off of large numbers of mites after the rapid increase was thought to be due to depletion of the food supply.

Field studies of the avocado brown mite, *Oligonychus punicae*, revealed that when the air was calm females began spinning and drifting activities soon after sunset. When weather permitted, these activities continued throughout the night. Mites drifting upward on rising air currents were trapped on aluminum strips, bordered with "Deadline," at the top of a 20-foot pole. For a period of 43 days all mites which drifted onto certain isolated terminals of an infested tree were removed at 24-hour intervals. Frequently the average number per leaf of females that drifted onto these isolated terminals was between $\frac{1}{4}$ and $\frac{1}{2}$ the average number per leaf on the rest of the tree.

Field studies of the six-spotted mite, *Eotetranychus sexmaculatus*, in an avocado grove revealed that in every respect the spinning and drifting activities were similar to those of the avocado brown mite.

A number of infestations of the two-spotted spider mite, *Tetranychus telarius*, on citrus trees in a greenhouse were studied. In no instance was this mite observed to spin down free from the leaves or to drift away from the infested plant.

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Chicken Louse and Mite Control with Malathion Formulations[1]

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Mohler *et al.* (1942) have estimated that the loss resulting from external parasites of poultry is about 85 million dollars per year. Van Es & Olney (1941) and Edgar & King (1950) have also indicated loss figures in poultry production due to attack by lice. Workers such as Bishopp & Wood (1939), Edgar *et al.* (1949), Furman & Bankowski (1949), and Quigley (1951) have reported on methods and materials to control one or more of the seven species of Mallophaga known to attack chickens in the United States. Vincent *et al.* (1954), Furman *et al.* (1955), and Kruiswyk & Brannon (1955) have discussed the use of malathion to control various poultry mites.

Because of the reported success of malathion in the control of ectoparasites of chickens it was felt desirable to carry out field tests under conditions found on the average Iowa farm using methods which could, and would, be easily employed by farmers. Therefore, in July of 1955 such field tests were begun.

METHODS AND MATERIALS.—Five buildings housing one-year-old laying flocks were selected for use in the tests. These hen houses were of frame construction containing wood roosts and dropping boards, straw or cob litter on the floor, and box-type nests holding straw nesting material, save one which had community type metal nests with a wire mesh floor. Four of the five hen houses each contained from 400 to 600 square feet of floor space. The fifth house contained 1,344 square feet.

The hens in all five houses were infested to a varying degree with three species of Mallophaga, namely, *Eomenacanthus stramineus* (Nitzsch), *Menopon gallinae* (L.) (shaft louse), and *Goniocotes gallinae* (DeG.) (fluff louse). Also, under the roosts, in the litter, and in cracks and crevices around the nests, countless thousands of the chicken mite, *Dermanyssus gallinae* (DeG.), could be found in all five houses. No other species of mite was present.

Counts of the lice were made on six randomly selected hens in each lot just prior to treatment and also at four subsequent posttreatment dates (Table 1). One-half of each bird was closely examined by parting the feathers. Particular attention was paid to the areas around the vent and under the wings. Because of their abundance no attempt was made to count the chicken mites and control of them was based on the presence or absence of live mites after treatment.

Table 1.—Malathion applied for control of lice and mites on chickens. Ames, Iowa, 1955.

	Average Number Lice per Bird						Chicken Mite Infestation		
		Days After Treatment			Per Cent Control			Per Cent Control	
Test	Pre-treatment	1	6	13	45	Initial	Total	Pre-treatment	Initial	45 Days
A[a]	34	4	16	20	—	88.3	0	Severe	100	100
B[b]	23	0	0	0	0	100.0	100.0	Severe	100	100
C[b]	38	0	0	0	0	100.0	100.0	Severe	100	100
D[c]	288	0	0	0	0	100.0	100.0	Severe	100	100
E[d]	92	0	0	0	0	100.0	100.0	Severe	100	100

[a] 1% malathion applied at 1 gallon per 1,000 square feet to walls, ceilings, roosts, and nests. Hens were shut out during spraying.

[b] Same as test A except hens enclosed in hen house during ceiling treatment.

[c] 4% malathion dust applied at 1 pound per 20 square feet to the litter and nests.

[d] Same as test D except not applied to nests.

Malathion was used both as a 1% spray and a 4% dust. The spray was mixed by adding 1 part of the 57% emulsifiable concentrate to 56 parts of water and was applied at the rate of 1 gallon per 1,000 square feet to walls, ceilings, roosts, and nest boxes as in residual fly control applications. In one of the houses sprayed, the chickens were shut out during the operation, while in the case of the other two houses, the chickens were kept in the house so that the spray drift fell on them. Immediately following treatment the surface of the feathers of the birds was damp to the touch but they were completely dry beneath the feathers.

The sprays were applied by the use of a nylon roller pump operated from a tractor power-take-off at 50 pounds pressure. The hand gun used for application was of the John Bean orchard-type.

Application of the 4% dust was begun using a rotary hand duster. However, this method proved so slow that the treatment was finished by broadcasting the material evenly over the litter with a coffee can. The dust was applied at the rate of 1 pound per 20 square feet.

RESULTS.—All treatments gave 100% control of the mite infestations for the entire 45-day observation period (Table 1). The louse infestations on the chickens were also controlled perfectly except in test A, where the spray was applied while all birds were excluded from the building (Table 1). Egg production increased markedly in the flocks where louse and mite control was achieved. In one flock the number of eggs per day increased from an average of 65 to an average of 95 in 3 weeks and continued at this level until the hens were sold to make room for a new flock of pullets. The other three farmers kept no accurate production records but estimated an increase of from 20 to 35%.

RESIDUE TESTS.—Fifteen hens were selected from a laying flock on a farm where no previous use of malathion had been made. Six of these were sprayed, 6 were dusted, and 3 left untreated as controls. The birds were treated by placing them in a sack, three at a time, then inserting the duster or spray nozzle in the neck of the sack. Approximately 5½ ounces of 1% malathion spray were applied to each of the six sprayed birds and ½ ounce of 4% dust to each of the dusted hens.

Four hours after treatment two hens from each of the treated groups and one from the control group were slaughtered and were immediately plucked, drawn, and quick-frozen. At 3 days after treatment and again at 7 days after treatment, the same procedures were followed. All birds were packed in dry ice and sent by air mail to

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Table 2.—Malathion residues in hens slaughtered at indicated intervals after treatment. Ames, Iowa, 1955.

DAYS AFTER TREATMENT	RESIDUES IN p.p.m.			
	Skin	Fat	Meat	Composite
1% emulsion sprayed directly on birds[a]				
0	4.0	0.9	trace	—
0	1.9[b]	—	—	—
0	2.0	—	—	—
3	0	0	0	—
7	—	—	—	0
4% dust applied directly on birds[c]				
0	0.5	trace	trace	1
3	1.3	trace	0	—
7	—	—	—	0

[a] Estimated 0.055 ounce actual malathion per bird.
[b] Cooked ½ hour before extracting.
[c] Estimated 0.02 ounce actual malathion per bird.

the American Cyanamid Company for malathion residue analyses. These analyses showed amounts of malathion present were highest in the skin under the conditions of these tests. In the case of those birds slaughtered 7 days after treatment, however, no residues of the insecticide were present (Table 2).

SUMMARY.—Field tests to determine the effectiveness of malathion in the control of the chicken mite, *Dermanyssys gallinae* (DeG.), and various species of Mallophaga on chickens were carried out during the summer of 1955. Sprays containing 1% malathion were applied to walls, ceilings, roosts, and nests of buildings housing laying flocks, at the rate of 1 gallon per 1,000 square feet of surface. Dusts containing 4% malathion were applied to the litter of two similar houses, at the rate of 1 pound per 20 square feet. Both the dust and spray applications gave complete control of the mites and lice encountered in these tests, except in one house where the hens were shut out while the spray application was being made.

Egg production records showed an increase of from 20 to 50% when the mites and lice were eradicated.

Residue analyses showed that no malathion remained in the hens slaughtered 7 days after treatment.

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Induced Lethargy and the Radiation Control of Insects[1]

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Recently there has been considerable national and international interest in the peacetime uses of atomic energy. One proposal under consideration has been the use of radiations for sterilizing foodstuffs and commodities. As Hassett & Jenkins (1952) point out, an exposure to penetrating radiations may solve the problem of infested products which cannot be subjected to heat treatment or insecticides. However, Sullivan & Grosch (1953) demonstrated that a holometabolous insect can tolerate extremely large doses of X-ray and furthermore that even an increase in longevity can be observed after irradiation. This phenomenon was detected in experiments with a braconid wasp, not an insect pest. Nevertheless, a little known paper by Davey (1919) was discovered which suggested the same prolongation of life in X-rayed *Tribolium*.

The wasp experiments were intentionally performed under starvation conditions. Whether a similar prolongation of life could be demonstrated under feeding conditions was not clear because of the great variability in length of life in routine culture of the wasp used (*Bracon hebetor* Say). Even with fruit flies which feed on yeasted media, Pearl & Parker (1924) performed experiments under starvation conditions in order to reduce the variation in the length of life. Although wasps have a number of biological features favorable for radiobiological research their ectoparasitic nature complicates life span

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studies. If the host caterpillar is not immobilized by prompt stinging it spins a web which may trap the wasp. In such an event the wasp dies in the struggle to free itself or the caterpillar may even kill the wasp by eating parts of it.

Fortunately the *Bracon* wasp is one which accepts pre-stung caterpillars as food. This occurred to us as a means of eliminating an important source of variability, the daily necessity for each experimental wasp to immobilize a host by stinging it. On this basis it has been possible to obtain data for the comparison, made below, between irradiated animals and controls kept with and without food.

MATERIALS AND METHODS.—A group of "nurse" wasps was maintained for the sole purpose of stinging and paralyzing host (*Ephestia*) caterpillars. At the start of the investigation each adult to be kept with food was given two paralyzed caterpillars. On each subsequent day the more flaccid caterpillar was replaced by a fresh pre-stung one. Stender dishes with ground glass covers were used as the most suitable containers for easy caterpillar handling. In addition, for behavior observations, the position of each individual wasp can be noted when the dish is given its routine 12-hour check.

Approximately 1,000 wild type virgin females of a highly inbred strain of *Bracon* stock number 33 were used for these experiments run in an incubator held at $30 \pm 1°$ C. An equal number of irradiated animals was placed in dishes with and without pre-stung caterpillars. Simultaneously, control wasps were carried with and without pre-stung caterpillars.

As in the earlier study (Sullivan & Grosch 1953), all radiation was delivered by the X-ray generator of the Marine Biological Laboratory, Woods Hole, Massachusetts. With proper precaution against overheating the wasps, this was operated in 187 kvp with a current of 28 ma to deliver 6,000 r per minute at the setting used. During irradiation, wasps were held in gelatin capsules.

RESULTS.—Life span for control and irradiated wasps as given by means and their standard errors is shown in table 1. On the average, at 30° C. normal wasps may be expected to live about 3 weeks ($20.92 \pm .59$ days) if host caterpillars are available for food. In contrast, starving wasps die after about 1 week ($7.18 \pm .10$ days) unless they have been exposed to a sizable dose of radiation. Irradiation may result in survival for additional hours or days. However, present evidence suggests that there is an optimum range of radiation for demonstrating this phenomenon at a given temperature. In the present case, 102,000 r was best. The effect with 180,000 r was not significantly higher than controls whereas, with this stock, 25,000 r was too low a dose since starving wasps did not survive equivalent controls.

Table 1.—A comparison of the mean longevity of female wasps, *Bracon hebetor* Say, when food is and is not available.

X-RAY DOSE (ROENTGENS)	LIFE SPAN IN DAYS	
	Food Available	Starvation Conditions
0	$20.92 \pm .59$	$7.18 \pm .10$
25,000	$11.51 \pm .59$	$6.73 \pm .42$
50,000	$12.34 \pm .34$	$8.93 \pm .35$
102,000	$11.09 \pm .32$	11.33 ± 1.01
180,000	$7.25 \pm .26$	$7.38 \pm .21$

In comparison with starvation results, the length of life observed when food was available is interesting and enlightening. After the higher doses of 102,000 and 180,000 roentgens the wasps apparently starved to death. Life span after these treatments was found to be essentially the same whether food was present or absent (comparison within treatment), and at death abdomens were flat. Undoubtedly the behavior observed for heavily irradiated wasps is contributory if not responsible for the results obtained. Strikingly characteristic is the lack of interest in food shown by these wasps even though it is present in abundance. In fact each wasp is found day after day standing at the same place in approximately the same position. We propose to call this completely abnormal behavior "radiation induced lethargy." It explains both the relatively early death of irradiated wasps in the presence of food and the increased life span under starvation conditions, the latter now shown to be a mere fraction of the survival time for feeding animals.

An interest in caterpillars was retained in wasps given the relatively lower doses of 50,000 and 25,000 roentgens. In particular, the rapid progressive dorso-ventral flattening typical of starvation was absent in wasps given 25,000 r. Nevertheless, it now becomes clear that heavy irradiation confers no advantage upon insects. In the present experiments, none of the irradiated wasps approached within two-thirds of the length of life of non-irradiated feeding controls. This finding is somewhat different from our experience at the same temperature with lower doses. After 7,950 r the average life span for feeding wasps was 17.36 ± 1.66 days when control life span was 19.80 ± 2.26 days. The shorter control life span as compared with the present experiments is due to several days of starvation before irradiation. These previously unpublished data were obtained in sterility studies (Grosch & Sullivan 1954) during which the females were observed to feed.

INSECT CONTROL.—From the standpoint of insect control, radiation induced lethargy influences survival in the undesirable direction. However this is offset by the fact that feeding stops. Thus, although immediate death of an adult insect may not be obtained without the delivery of several hundred thousand roentgens, less massive doses in the neighborhood of 100,000 r may be expected to halt the damage done by adults. Furthermore, a dose as large as this is well above the radiation required for sterilizing all adults (Grosch & Sullivan 1954) as well as for the killing of immature forms (Clark & Mitchell 1951). Although Hassett & Jenkins (1952) ran experiments with cobalt 60 gamma rays at levels up to 257,600 r, they suggested a relatively light dose of 65,000 r for routine insect control. Our observations indicate that some insects may continue to feed for a while after this dose.

Furthermore, temperature is a variable which has not yet been adequately emphasized in papers on the radiation influence upon insect life span. Experiments run at 5 degrees lower than the present showed wasps of the same stock living half again as long (Grosch & Sullivan 1953). Even more striking is the demonstration (Grosch

1950) that the average survival for starving *Bracon* females is 10 weeks at 5° C. and that a few individuals can survive for more than 3 months at this temperature. Therefore, contraindicated is storage of infested substances at cool temperature after irradiation.

DISCUSSION.—Radiation induced lethargy is probably the milder expression of a phenomenon well known to investigators who have examined insects directly after very high doses of ionizing radiations. Hassett & Jenkins (1952) have called it "temporary knockdown" and Heidenthal (1945) used the term "sluggishness." *Lyctus*, one of the pests used by the former, apparently reacts in such a way at relatively low doses, while it takes more than 150,000 r for a good demonstration in *Habrobracon* (Heidenthal 1945).

In the present experiments, a knockdown was observed after the highest dose. Upon removal from the X-ray generator after 180,000 r, all wasps were on their backs or sides. Many showed slight twitchings of the legs. However, within minutes all these wasps were back on their feet and would move normally if touched. If undisturbed, the irradiated wasps remain quiescent and hence do not use up their stored reserves as rapidly as normal, starving wasps.

From the clinical standpoint, the lethargy noted in irradiated insects may be a parallel of the anorexia and asthenia of radiation sickness in humans (Bacq & Alexander 1955) as well as the apathy described for lower mammals after whole body irradiation (Trum 1953). In contrast to the insect, however, such effects are experienced by the mammal after much less radiation, and a dose of about 8,000 r which has little effect on wasp life span is fatal to mammals within hours.

Bacq & Alexander (1955) have reviewed the pathological biochemistry of irradiated organisms, and conclude that little change in general metabolism is found with the minimum 100% lethal dose except just before death. Recent findings on *Drosophila* by King & Wilson (1955) are consistent with this view. Because of the failure to demonstrate marked biochemical disturbances after total-body irradiation it does not seem profitable to speculate on the nature of induced lethargy beyond mentioning that knockdown and twitchings suggest an influence on the nervous system. In young mice, Brunst (1954) has produced roentgen paralysis which is not lethal to the organism. In fact the various organ systems other than the nervous system continued to function adequately for 10 minutes in one of his mice. Until recently, adult nervous tissue has been regarded as highly radioresistent, but mesoglia and receptor units have proved very sensitive (Bacq & Alexander 1955). Also brain has now been demonstrated as the only mammalian tissue which increases in dry weight after irradiation (Rugh 1954).

SUMMARY.—After massive doses of radiation, adult insects as exemplified by a wasp, are lethargic. This explains their increased life span under starvation conditions. However since they show no interest in feeding, heavily irradiated insects (100,000 r or more) provided with food, die long before untreated feeding siblings. The temperature at which life span is studied is an important factor particularly if insect control in stored products is contemplated. A parallel is suggested between insect lethargy and symptoms of radiation sickness in mammals. Speculation implicates the nervous system.

If the presence of non-feeding, sterile adults is not objectionable, doses of radiation merely adequate to induce lethargy may be a more feasible approach to insect "control" than fast-killing doses.

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Thrips Control on Peanuts with Granulated Insecticides[1]

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Thrips have caused sufficient damage to peanuts in North Carolina to create a demand for control measures. The economic importance of thrips in peanut production, however, is not easily evaluated. Injury by thrips to peanuts was reported more than 35 years ago in Florida by Watson (1918). However, little heed was paid to their importance on peanuts until Poos (1941) established that thrips could be held responsible for the condition known as peanut "pouts."

Injury by thrips to peanuts occurs mainly when the insects feed on the upper epidermis of the unopened terminal leaflets. The species of thrips found associated with leaf injury are *Frankliniella fusca* (Hinds) and *F. tritici* (Fitch). Only *F. fusca* has been reared from nymphs found on injured peanut plants by Poos (1941). The flower thrips, *F. tritici* has been found in only small numbers on damaged peanuts and Watson (1916) pointed out that this species breeds only in blossoms. The tobacco thrips, *F. fusca* breeds on peanut foliage and is largely responsible for thrips injury to peanuts.

Attempts by Poos (1941) to reduce thrips injury by the use of a tartar emetic-brown sugar spray in Virginia were unsuccessful. However, Poos (1945) reported that the use of DDT as a dust, spray (suspension) or aerosol gave good thrips control. A tartar emetic-brown sugar spray was less effective while pyrethrins and sulfur were both ineffective. Yields, based on green weights on plants, were increased by approximately one-third in plots in which thrips were controlled. Comparison of actual peanut yields was not possible since the work was conducted in Maryland where climatic conditions do not permit maturity of all pods formed.

Further work in Maryland and Virginia (Poos *et al.*, 1947, Poos & Dobbins 1951) substantiated previous results with DDT. BHC as a dust or spray (emulsion) gave good thrips control even though it lacked residual effectiveness. Ryania spray was ineffective. Though not considered significant, there was an average increase of over 15% in nut yields in plots treated with DDT spray for thrips control. Control of these insects was reported to be more important on soils of low fertility than on more fertile land.

Results of experiments conducted in Alabama during the period from 1946 to 1950 (Arant 1954) indicated that DDT sprays and dusts, toxaphene and BHC were very effective against thrips; sabadilla gave fair control, while sulfur, rotenone and nicotine were ineffective. No consistent yield gains were attributed to applications of DDT or toxaphene dust for thrips control.

Tests in Virginia involving the use of demeton soil drenches (Howe & Miller 1954) showed thrips control evident at the higher dosages tested. However no significant yield differences resulted from the insect control obtained and apparent phytotoxic effects were observed.

Research, reported here, on the means of controlling thrips on peanuts in North Carolina was conducted during the years 1953 and 1954. The initial objective of the work begun in 1953 was to evaluate the damage to peanuts caused by thrips. In pursuing this aim, the necessity for controlling other insects led to the discovery that thrips populations appeared to be affected more by granulated insecticides applied for the control of soil insects than by additional foliage treatments made specifically for the control of thrips. The major objective of subsequent work was, therefore, to evaluate the effectiveness of certain granulated insecticides against the tobacco thrips by comparing them with one another and with insecticides of both known and unproven value applied as dusts or sprays.

MATERIALS AND METHODS.—In 1953, experimental work was conducted in a field, located on the Upper Coastal Plain Experiment Station near Rocky Mount, N. C. The soil in the field was fairly "heavy" and quite variable in fertility. This variability was emphasized when the intended application of muriate of potash was not made and several degrees of potash deficiency were observed.

Plots were arranged in a randomized complete block design replicated three times. Whole plots, treated in various ways for the control of the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, were divided into subplots, one of which was to be chemically treated for thrips control.

The plots were treated[3] with granulated Attaclay formulations,[4] with the exception of one set treated with granulated tobacco, as indicated:

2% aldrin applied with a So-Rite fertilizer distributor at 2 lbs. per acre.
10% aldrin applied with a power duster at 1.8 lbs. per acre.
10% aldrin on tobacco applied with a power duster at 2.1 lbs. per acre.
20% toxaphene applied with a So-Rite fertilizer distributor at 24.3 lbs. per acre.
20% toxaphene applied with a So-Rite fertilizer distributor at 14.6 lbs. per acre.
5% dieldrin applied with a power duster at 0.6 lb. per acre.
5% heptachlor applied with a power duster at 1.5 lbs. per acre.
Untreated

All soil treatments were made on May 22. Those treated with a duster were six rows wide; those treated with the fertilizer applicator were five rows in width. Plots ranged in area from 0.20 to 0.28 acre, the total acreage involved in the test being approximately 6 acres. Subplots were 125 feet in length, and each included an area of 0.05 acre.

Pre-DDT-treatment thrips counts were made on June 15 through June 18 and subplots were treated with 5% DDT dust.[5] This material was applied on June 18 with a

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[3] All rates of application refer to amount of technical insecticide applied.

[4] All granulated formulations on clay and tobacco were prepared and furnished by the Virginia-Carolina Chemical Corporation, Richmond, Va.

[5] DDT formulations furnished by Taylor Chemical Co., Aberdeen, N. C.

rotary hand duster at the rate of 0.7 pound per acre. Thrips populations were estimated on the basis of the number of thrips (nymphs and adults) per 10 terminals plus the last "open" leaf. Three post-treatment counts were made in all DDT-treated subplots. The first was made on June 29 to 30; the others were made on July 8 to 10 and July 20 to 22.

Soil insect population counts were made in all plots prior to treatment and once in every 10-day period from June 2 through September 15. Yields were estimated by digging, stacking, picking and weighing the entire crop in each subplot and converting to a per-acre figure. Analyses of variance were run on the insect population and yield data.

Field work in 1954 was conducted in a large portion of a 3-acre field located, as in 1953, at the Upper Coastal Plain Experiment Station near Rocky Mount, N. C. Peanuts of the NC-2 variety were planted on April 29 in 3-foot rows. Cold weather following planting resulted in slow emergence and an uneven stand.

The field was divided into two tiers of plots, each plot six rows wide. One tier contained plots of equal length, each plot including an area of 0.05 acre. The other tier contained plots of unequal size due to the triangular shape of the field. The total area involved in the test was 2.8 acres.

Each tier of plots was regarded as a separate test. However, to facilitate application with farm-type equipment, treatments in the two tests were so aligned that plots in the two tiers receiving the same soil treatment were located end to end. Treatments were randomized in each block, the arrangement in the two tiers being similar due to the alignment described above. The treatments compared in the two tiers were as follows:

(1) 10% aldrin on granulated Attaclay at 3 lbs. per acre.
10% aldrin on granulated tobacco at 2.3 lbs. per acre.
5% dieldrin on granulated Attaclay at 1.2 lbs. per acre without agitation.
5% dieldrin on granulated Attaclay at 1.5 lbs. per acre.
5% heptachlor on granulated tobacco at 1.4 lbs. per acre.
5% heptachlor on granulated vermiculite[6] at 1.5 lbs. per acre.
Toxaphene[7] spray emulsion at 1.5 lbs. per acre.
10% toxaphene on granulated vermiculite at 20 lbs. per acre.
1% parathion[8] dust at 0.4 lbs. per acre.
Untreated.

(2) 10% aldrin on granulated Attaclay at 3 lbs. per acre.
10% aldrin on granulated tobacco at 2.3 lbs. per acre.
5% DDT dust at 1 lb. per acre.
Dieldrin as granulated plus DDT spray emulsion at 0.75 lbs. per acre.
5% heptachlor on granulated tobacco at 1.4 lbs. per acre.
5% heptachlor on granulated vermiculite at 1.5 lbs. per acre.
DDT spray emulsion at 0.75 lb. per acre.
20% toxaphene on granulated vermiculite at 20 lbs. per acre.
Malathion[8] spray emulsion at 1 lb. per acre.
Strobane[9] spray at 1.5 lbs. per acre.

All granulated formulations were applied with a 6-row power duster on June 1 and 2. The agitator was used in all treatments except as noted above. Dust treatments were made on June 23 with a rotary hand duster; sprays were applied on the same date using a Boyett self-propelled sprayer.

Table 1.—Thrips populations in peanut plots treated with various granulated formulations of insecticides and a single application of DDT dust near Rocky Mount, N. C., 1953.

Soil Treatment[a] (Pounds per Acre)	Average Number of Thrips per 10 Terminals			
	Before DDT Treatment[b]	After DDT Treatment		Seasonal Average in DDT-treated subplots
		Treated subplots	Untreated subplots	
Applied with fertilizer distributor:				
Toxaphene, 24.3	21.5	2.0	10.3	10.0
Toxaphene, 14.6	23.8	1.7	8.7	10.2
Aldrin, 2.0	0.4	0	1.0	1.3
Untreated	49.8	4.3	11.3	18.6
Applied with a power duster:				
Aldrin, 1.8	3.7	0	3.0	2.7
Aldrin on tobacco, 2.1	2.5	1.0	5.0	1.5
Dieldrin, 0.6	13.2	0.3	5.3	4.1
Heptachlor, 1.5	20.2	3.3	12.7	8.6
L.S.D. at 5% level	15.08			5.32
at 1% level	20.94			7.38

[a] All formulations on granulated Attaclay unless otherwise designated.
[b] Average for both subplots.

Pretreatment counts were made during the period May 27 through June 1 and the thrips present were identified as *Frankliniella fusca* (Hinds). Post-treatment counts were made on June 30 through July 1, July 15 and 16, and July 29 and 30. Populations were estimated in the same manner as in 1953.

Plots treated with the organic phosphates, parathion and malathion, received an additional application of parathion on granulated tobacco at the rate of 1.1 lbs. of parathion per acre. This treatment was made on July 9, limiting the observations on the effects of the foliage treatments to a single reading.

Soil insect population counts were made in all plots prior to treatment and once every 2 weeks from July 8 through September 8. Yield estimates were made as in 1953 and analyses of variance were run on insect population and peanut yield data.

RESULTS.—In 1953 thrips counts made prior to treatment with DDT, presented in table 1, showed differences which appeared to be directly related to the soil treatments made 3 weeks previously.

Plots treated with granulated aldrin were almost free of thrips. The dieldrin treated plots had a somewhat higher infestation. Heptachlor and toxaphene treated plots had the highest populations of the treated plots but even in these, there were only half as many thrips as in the untreated plots.

The average seasonal thrips populations in the various plots, based on three post-treatment counts and the pre-DDT treatment counts, are also presented in table 1. Since all subplots represented by these data were treated alike with DDT, the population figures are largely an indication of the effects of the soil treatments. Though

[6] Vermiculite formulations furnished by the Zonolite Co., Travelers' Rest, S. C.
[7] Toxaphene dusts and spray concentrates furnished by the Hercules Powder Co., Wilmington, Del.
[8] Furnished by the American Cyanamid Co., New York, N. Y.
[9] Furnished by B. F. Goodrich Chemical Co., Cleveland, O.

complete thrips data were not taken on subplots not treated with DDT, due to a lack of available time, those data taken suggest that the populations would have been generally higher and the differences between treatments greater if complete counts had been made in these plots throughout July.

Counts made during the second week after the application of DDT dust, also presented in table 1, show a tendency for this insecticide to reduce the differences in thrips populations as affected by soil treatments.

Thrips infestation in plots treated with various granulated insecticides and population reduction in these plots with and without the additional application of DDT dust are shown in table 2. The effectiveness of this material is best indicated by the data from plots receiving no soil treatment and from those treated with toxaphene. In these the reduction in population in DDT-treated subplots exceeded 90%. Actually by this time, plant development (an increase in the number of "terminals") and the maturation and emigration of thrips had resulted in a reduction of almost 75% in the number of thrips per 10 terminals in the completely untreated check. Where populations had already been severely reduced by soil treatment with aldrin, the effects of DDT could not be accurately measured, though in all cases where thrips were present they were reduced in numbers after the use of DDT.

Observations made during the season and extreme variation in peanut yields obtained in various parts of the test field indicated that experimental design was not compensating for the existing soil variation. Not only was there a highly significant difference between yields in the various replicates and no significant yield difference between soil treatments, but great differences were observed within replicates which could not be associated with the treatments.

The only difference in yield means which was just significant at the 5% level, was that between the DDT-treated and untreated subplots. Since the higher yields were obtained on subplots not treated with DDT, it was recognized that this and possibly other insecticides used might have a direct effect on yield not associated with insect control.

The distribution of populations of other insects, particularly those inhabiting the soil, was very irregular and not considered a random distribution. Considering the complicating factors referred to above, it was not possible to show any simple association between yield and thrips control.

A summary of data on thrips populations as affected by the various treatments used in the 1954 tests is given in table 3. Seasonal average populations as reported here include only the three posttreatment counts. Each tier of plots was analyzed separately for most accurate comparisons. In order to indicate the relative effectiveness of treatments in both tiers, mean population figures have been determined for all plots treated alike and all treatment means are presented for comparison. Percentage reduction is based on pretreatment populations. The irregularity in plot size and two missing plots in the second tier made the sampling less accurate and comparisons more difficult in these plots.

Granulated toxaphene was ineffective as a seasonal control against thrips. Granulated heptachlor formulations gave relatively poor thrips control; the vermiculite carrier giving consistently poorer results than the tobacco carrier. The concentration of the two formulations was the same, 5%. Strobane spray also gave relatively poor results. The remaining materials in the formulations used gave favorable results against thrips. Granulated dieldrin gave varying results generally indicating a fair degree of control. The organic phosphates also gave a fair degree of control, but evaluation could not be accurately made,

Table 2.—Thrips population reduction in peanut plots treated with various granulated formulations with or without an additional application of DDT dust near Rocky Mount, N. C., 1953.

Treatment[a]	No. of Thrips/10 Terminals[b]		Per Cent Reduction	
	Before DDT Treatment	After DDT Treatment	DDT-Treated	No Foliage Treatment
Applied with fertilizer distributor:				
Toxaphene at 24.3 lbs./A (only)	18.0	10.3		42.8
(plus DDT dust later)	25.0	2.0	92.0	
Toxaphene at 14.6 lbs./A (only)	20.3	8.7		57.1
(plus DDT dust later)	27.3	1.7	93.8	
Aldrin at 2.0 lbs./A (only)	0.7	1.0		—
(plus DDT dust later)	0	0	—	
No soil or foliage treatment	45.0	11.3		74.9
(plus DDT dust later)	54.7	4.3	92.1	
Applied with power duster:				
Aldrin at 1.8 lbs./A (only)	3.7	3.0		18.9
(plus DDT dust later)	3.7	0	100.0	
Aldrin on tobacco at 2.1 lbs./A (only)	3.0	5.0		—
(plus DDT dust later)	2.0	1.0	50.0	
Dieldrin at 0.6 lb./A (only)	17.7	5.3		70.0
(plus DDT dust later)	8.7	0.3	96.6	
Heptachlor at 1.5 lbs./A (only)	21.3	12.7		40.4
(plus DDT dust later)	19.0	3.3	82.6	

[a] All formulations on granulated Attaclay unless otherwise indicated.
[b] Average for three replicated plots.

Table 3.—Thrips control in experimental plots at the Upper Coastal Plain Experiment Station near Rocky Mount, N. C., 1954.

Treatment (Pounds per Acre)	Seasonal Average No. Thrips per 10 Terminals			Per Cent Reduction[a]
	Tier I	Tier II	Both Tiers	
DDT dust, 1		2.5	2.5	92.9
DDT spray, 0.75		4.2	4.2	83.0
Aldrin on granulated Attaclay, 3	2.9	4.7	3.8	93.3
Aldrin on granulated tobacco, 2.3	3.2	5.2	4.2	87.4
Toxaphene spray, 1.5	3.9		3.9	90.7
Parathion on granulated tobacco, 1.1			4.8	89.5
Parathion dust, 0.4	5.3		5.3	87.7
Malathion spray, 1		6.0	6.0	87.0
Granulated dieldrin without agitator, 1.2	4.7		4.7	87.0
Granulated dieldrin with agitator, 1.5	5.4		5.4	83.2
Granulated dieldrin, 1.5 plus DDT spray, 0.75		6.0	6.0	81.4
Heptachlor on granulated tobacco, 1.4	9.2	6.3	7.8	79.7
Heptachlor on granulated vermiculite, 1.5	10.4	7.0	8.7	69.5
Strobane spray, 1.5		8.0	8.0	75.5
Toxaphene on granulated vermiculite, 20	13.6	19.1	16.3	64.8
Untreated	17.5		17.5	46.4
L.S.D. at 5% level	4.73	7.42		
at 1% level	6.48	10.22		

[a] Based on pretreatment populations.

because all plots involved received two different applications of a phosphate material during the period of thrips activity. Granulated aldrin, DDT dust, and toxaphene spray gave good control. DDT spray gave varying results from good to fair. Aldrin on granulated Attaclay and DDT dust consistently gave the best results. Both aldrin formulations were of the same concentration, 10%.

Though the soil variation evident in the 1953 test was not of the same magnitude in the field used in 1954, variation in distribution of several important insect pests and the possibility of a direct effect of the insecticides on yield again prevented demonstration of a simple association between yield and thrips control.

DISCUSSION.—The results with granulated formulations of insecticides against thrips in 1953 and 1954 showed that aldrin, dieldrin and heptachlor in that order gave good to fair control of the tobacco thrips on peanuts, while toxaphene was relatively ineffective. The mode of action of these materials in reducing thrips populations is not clearly understood. Though the insects may be killed by coming in contact with the insecticide on the surface of the soil, it appears more likely that thrips control is associated with the capacity of the toxicant to act as a fumigant and the amount of volatile toxicant released over the period during which these insects are most active in the peanut fields.

Though differential control of thrips populations by means of various insecticides applied to the soil and/or peanut foliage can be demonstrated, the economic value of such control is difficult to ascertain. The problem is complicated by soil variability, the presence of irregularly distributed (and in some cases more injurious) pests, as well as by possible direct effects of the insecticides used.

A simple association of peanut yields and thrips control appears to be possible only on very uniform soil and with all other destructive insects controlled. Unless such a situation can be utilized, it is evident that in order to make any logical estimate of thrips damage, an analysis procedure, which will take into account all of these complicating factors, must be employed. Such a method was developed for utilizing the data taken in this test in evaluating thrips injury. However, discussion of the technique is considered beyond the scope of this report.

SUMMARY.—Experiments conducted under the dry conditions prevailing in North Carolina's coastal plain during the 1953 and 1954 growing seasons and dealing with thrips control on peanuts revealed:

1. The most effective control measures against thrips were DDT dust at 1 pound per acre, granulated aldrin at 2.3 to 3.0 pounds per acre, toxaphene spray at 1.5 pounds per acre and DDT spray at 0.75 pound per acre.

2. Materials which gave promising results against thrips were granulated dieldrin at 1.2 to 1.5 pounds per acre, granulated parathion at 1.1 pounds per acre, parathion dust at 0.4 pound per acre and malathion spray at 1 pound per acre.

3. Granulated heptachlor at 1.4 to 1.5 pounds per acre and Strobane spray at 1.5 pounds per acre were less effective.

4. Granulated toxaphene at 20 pounds per acre was relatively ineffective against thrips.

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Experiments on Equipment and Materials for Spraying Alfalfa for Insects[1]

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This is a report of 2 years' work on different types of spray equipment for applying insecticide to alfalfa for control of pea aphid (*Macrosiphum pisi* (Harr.)), meadow spittlebug (*Philaenus leucophthalmus* (L.)), and alfalfa weevil (*Hypera postica* (Gyll.)), and 1 year's work on the effectiveness of several insecticides against the above insects. In 1954 and 1955 two boom designs were tried, the sweep type usually used for weed and insect control, and broadcast type as described by Ditman & Burkhardt (1952) for control of aphids on peas. The latter boom was tested with hollow cone nozzles in 1954 and with flat spray nozzles both years. The amounts of water were varied from 10 to 50 gallons per acre and spray pressures of 40 and 100 p.s.i. were used. The comparative effectiveness of heptachlor, dieldrin, BHC, and malathion was tested in one experiment in 1955. The 1954 work was done in Carroll County and the 1955 work in Howard County, Md.

Method of Application.—*1954 Experiment.*—Eight treatments (table 1) were made on April 19 to plots of alfalfa in randomized blocks approximately 80×30 feet which were replicated four times. Heptachlor emulsion (2 pounds per gallon) was applied at the rate of 1 pint (4 oz.) per acre in all treatments. Results are based on insect population counts. Spittlebug nymphal populations were estimated by counting nymphs on 40 plant stems, while spittlebug adults, alfalfa weevils, and aphids were determined by sweeping. Results are given in table 1.

1955 Experiments.—(a) Six treatments (table 2) were applied to alfalfa plots on April 19 when the plants were 8 to 10 inches high. Each plot was 130×30 feet and all treatments were randomized in blocks replicated four times. Since the spittlebug population was extremely low in these plots (4 on 40 unsprayed stems, May 3) no records are given of this insect. Alfalfa weevil and aphid populations were determined by sweeping. Results of treatments are given in table 2.

(b) Four insecticides, all in the emulsified form (table 3), were tested against the aphid and alfalfa weevil. The various concentrates were of the following strength: BHC, 11%; dieldrin, 16%; heptachlor, 23%; and malathion, 55%. All insecticides were applied in 25 gallons of water per acre at 100 p.s.i. with the weed type boom equipped with flat spray nozzles number 65015. The results are given in table 3.

Discussion.—The three species of insects concerned with alfalfa in these experiments differ in their method of feeding and where they feed on the plant. The spittlebugs, sucking insects, when young feed near the base of the plants and in order to reach them the sprays must penetrate the upper foliage. The flat type spray nozzle on a sweep boom would appear best for this purpose. The flat nozzles give a rather long penetrating fan of spray and arranged in a sweep boom give very even distribution of the spray over the area covered.

The pea aphids feed over all of the plant but are usually concentrated on the growing tips. As aphids are sucking insects also, it would appear that thorough coverage of the plant is necessary. This should be best accomplished by the pea-spraying broadcast boom equipped with cone nozzles (Ditman & Burkhardt 1952). On this boom the nozzles are arranged in two rows, one row directed forward 45 degrees and to the right 45 degrees, with the second row of nozzles directed backward 45 degrees and 45 degrees to the left, so that all plants are effectively sprayed from two sides.

The alfalfa weevils, both adults and larvae, feed mostly on the growing tips of the plants but since they are chewing insects, and consume both surfaces of the leaves, complete plant coverage, as needed for pea aphid, should not be necessary. Both types of nozzles and both types of booms should be satisfactory for this insect.

For maximum effectiveness the pea-type boom equipped with flat spray nozzles would appear to be best, giving good coverage by striking the plants from two sides and at the same time penetrating the foliage to give

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Table 1.—Effectiveness of different types of equipment in spraying[a] alfalfa, 1954.

Test	Boom Type	Nozzle Type[b]	Pounds per Sq. In.	Gallons per Acre	Insect Counts on Dates Examined: April 23 Spittlebug nymphs[c]	April 23 Aphids[d]	April 23 Weevil larvae[d]	May 3 Spittlebug nymphs[c]	May 3 Aphids[d]	May 3 Weevil larvae[d]	May 26 Spittlebug adults[d]	May 26 Weevil larvae[d]
a	Weed	650067	100	10	6.8	91	0	10.7	62	.5	2.8	86
b	Weed	65015	100	25	5.8	134	0	7.7	80	.2	.6	82
c	Pea	D-2-13	100	25	7.5	95	0	11.7	85	.7	1.2	80
d	Pea	650067	100	25	10.8	91	0	21.4	81	.0	1.2	107
e	Pea	D-2-25	100	50	6.4	56	0	13.2	72	.5	2.1	88
f	Pea	6501	100	50	9.9	64	0	11.3	69	.2	1.0	92
g	Weed	6503	40	50	4.9	103	0	8.8	59	.2	.7	105
	Control	—	—	—	22.0	141	2	64.6	46	8.7	4.0	97
	L.S.D. at 5% level		—	—	4.9	51	ns	10.8	ns	4.3	1.2	ns

[a] All plots sprayed with heptachlor, 1 pint of 2 lb. per gal E.C. (0.25 lb.) per acre.
[b] Spraying Systems Co., Bellwood, Illinois.
[c] Number of nymphs per 10-plant sample.
[d] Number insects per sweep.

Table 2.—Effectiveness of different types of spray equipment in spraying alfalfa,[a] 1955.

Test	Boom Type	Nozzle Type	Pounds Pressure Used	Gallons per Acre	Insects per Sweep on Dates Examined: April 28 Aphids	April 28 Alfalfa Weevil Adults	April 28 Alfalfa Weevil Larvae	May 5 Aphids	May 5 Alfalfa Weevil Adults	May 5 Alfalfa Weevil Larvae
a	Weed	65015	100	25	6.5	.8	.02	33.8	2.6	.8
b	Pea	650067	100	25	3.7	1.5	.00	26.7	2.6	.8
c	Weed	6502	40	25	21.8	1.7	.00	64.4	2.9	2.7
d	Weed	6501	100	12.5	20.1	2.0	.00	65.2	2.1	1.5
e	Weed	6502	100	50	7.7	1.1	.00	19.0	2.5	.1
	Control				116.6	1.2	.25	133.9	1.3	7.1
	L.S.D. at 5% level				42.6	0.3	ns	41.1	ns	3.0

[a] All plots sprayed with 11% BHC, 1 quart of emulsion concentrate (0.25 lb. gamma isomer) per acre.

satisfactory coverage for spittlebug control. It is difficult in a single experiment to test these hypotheses since these insects do not respond equally well to the same insecticides and also since they do not always occur in abundance at the same time.

RESULTS.—Results of the 1954 experiments (table 1) show that all methods of application gave significant reductions of spittlebug nymphs; that contrary to expectations, the pea boom equipped with hollow cone nozzles (c and e) gave as good control of spittlebug as the fan-nozzled weed type boom.

On aphids the pea-type boom tended to be more effective than the fan-nozzled weed booms used. With the pea boom, spray applied at the 50 gallon per acre rate (e and f) was slightly superior to spray applied at the 25 gallon rate (c and d). Only with the pea boom at the 50 gallon per acre rate and 100 pounds pressure (e and f) were aphid populations significantly (at 5%) reduced compared with those of untreated plots (April 23 counts).

There were insufficient alfalfa weevil larvae present to evaluate treatments on April 23, but counts on May 3 showed all treatments significantly reduced larval populations and that there was no significant difference between any of the treatments on that date. This single application of insecticide (April 19) did not protect alfalfa from late alfalfa weevil infestation, as the May 26 counts show.

In 1954 contour strips of alfalfa adjacent to the experimental plots were sprayed with BHC on the same day plot treatments were made. The pea boom equipped with number 650067 nozzles was used and the 11% BHC emulsion was applied at the rate of 1 quart (4 oz. gamma isomer) in 25 gallons of water per acre. On May 3 there were 0.5 spittlebug nymphs per 10 plant sample in the BHC strips as contrasted with 21.4 per 10 plants on the heptachlor plots sprayed with the same equipment (d).

Table 3.—Effectiveness of several insecticides[a] against the pea aphid and alfalfa weevil on alfalfa.

Insecticide	Rate per Acre: Pints	Ounces	Insects per Sweep on Dates Examined: April 28 Aphids	April 28 Weevils Adults	April 28 Weevils Larvae	May 5 Aphids	May 5 Weevils Adults	May 5 Weevils Larvae
Heptachlor	1.60	6	188.4	0.0	0.00	165.4	0.0	0.0
Dieldrin	1.33	4	110.6	.0	.02	235.2	1.0	.1
BHC	2.00	4	9.9	.5	.00	39.1	6.7	.2
Malathion	1.50	15	4.9	.3	.17	22.7	4.0	5.0
Control			175.4	.6	.60	251.9	0	8.9
L.S.D. at 5% level			3.7[b]	ns	ns	7.5[b]	ns	4.1

[a] Applied April 21, 1955, with a weed type boom equipped with fan nozzles in 25 gallons of water per acre.

[b] Calculated for BHC and malathion treatments only, since these were the most effective insecticides against the aphids.

In the 1955 method-of-application experiments (table 2) all treatments gave a significant reduction of aphids. The greatest reduction (April 28 counts) resulted from the use of the pea-type boom equipped with fan spray number 650067 nozzles (b). However, serious difficulty was experienced because of stoppage and these small aperture nozzles do not appear practical for farm use. All treatments made with 100 pounds pressure and 25 to 50 gallons per acre were effective. Where either pressure or gallonage (c and d, respectively) was reduced, pea aphid control dropped.

Numbers of alfalfa weevil larvae were considerably reduced by all treatments in 1955, though there were no significant differences between treatments either for adult weevils or larvae. The treatment at 40 pounds pressure gave poorer results than any of those applied at 100 pounds pressure.

Results of the 1955 insecticide experiments (table 3) showed both BHC and malathion effective against the pea aphid, malathion giving a significantly greater reduction than BHC. Dieldrin and heptachlor treatments were ineffective against this insect.

Heptachlor and dieldrin out-performed BHC and malathion (table 3) against alfalfa weevil adults and all materials but malathion were effective against larvae. The May 5 differences on larvae were significant.

SUMMARY.—Alfalfa was sprayed in 1954 and 1955 in replicated plots 30 feet wide using different arrangements of spray booms and nozzles and different quantities of water, keeping the rate of insecticides per acre constant. In 1955 in another experiment four insecticides were used with one equipment arrangement. Comparisons were made on resulting insect populations.

Against the pea aphid in 1954, using heptachlor emulsion, only the pea broadcast boom, designed to spray plants from two sides, with 50 gallons of spray per acre reduced the population significantly. In 1955, using BHC emulsion, a boom of the same design gave greatest reduction of pea aphid. Against the nymphs of the meadow spittlebug and alfalfa weevil there was no significant difference between the weed type boom equipped

with flat nozzles and the pea broadcast boom equipped with hollow cone nozzles. In 1955 a reduction of spray pressure from 100 to 40 p.s.i. and a reduction of water from 25 to 12.5 gallons reduced the effectiveness of treatments against both the pea aphid and alfalfa weevil. Of the insecticides tested, malathion was most effective against the pea aphid, BHC most effective against the meadow spittlebug, and heptachlor most effective against the alfalfa weevil.

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Determination of Insecticide Residues in Soil by Using *Drosophila*[1]

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Biological assay indicated that significant amounts of insecticides remained in soil 4½ years after treatment. Seven insecticides were applied to soil in relatively large quantities to determine the effect on growth of peach trees. The amount of insecticide applied was the quantity that might be expected to be used in 10 years in a peach spray program. Typical soils in two principal peach growing areas of Arkansas were used. Composite soil samples were taken from the treated plots and the amount of insecticide remaining was determined by using 1-day-old adults of *Drosophila melanogaster* Meig. The methods employed in rearing, handling and using the flies, and the results obtained are presented.

The flies were reared in 1-quart wide-mouthed fruit jars. Canned pumpkin was used as the rearing medium. The jar lid was replaced with a single thickness of paper toweling. One to two inches of pumpkin was placed in the bottom of the jar and a little dry yeast worked into the surface. The flies to be used as breeding stock were shaken down into a 30×100-mm. vial, quickly emptied into the jar and the towel lid replaced. From 50 to 100 flies were used to stock a jar. The flies were removed after 1 to 3 days, depending on how soon the desired number of eggs were deposited. The yield of flies from a breeding jar was increased if several wadded strips of paper toweling were pressed into the pumpkin 3 to 5 days after the jar was set up. This resulted in making the pumpkin less moist and increased the surface area.

Flies were removed from the rearing jars into 1-quart seal-rite containers which had the bottom replaced with fine mesh screen (Fisher & Smallman 1954). The carton was fitted over the top of the rearing jar and the flies moved upward and toward the source of light into the carton. The top of the carton was made of wood with a ⅞-inch hole into which a feeder was placed. The flies were fed canned pumpkin plus yeast. Flies for testing were held for 24 hours after removal from the breeding jars. Flies were removed from the holding cages by placing a 30×100-mm. vial over the hole in the top of the holding cage. The vial was then stoppered with a cottom plug. The same type vial was used for exposing the flies to the material containing the insecticide. Flies were aspirated into the testing vials. The cork was placed in the testing vial by shaking the flies down in the vial and replacing the aspirator with the cork in one quick motion.

The soil samples to be tested for insecticide residues were obtained with a soil auger taking approximately 50 borings to a depth of 6 inches. The insecticides had been applied as wettable powders to the soil surface and disked in. The effect of these materials on the growth of newly set peach trees has been reported (Wylie 1952). The soil obtained with the auger was mixed to obtain a composite sample for each treatment.

To prepare the soil samples for testing for residues, the lumps were broken up and screened to remove small stones. Five grams of air-dried soil was then weighed into each of four vials. The soil was moistened with 2 ml. of an aqueous solution containing 25% by volume of white Karo. A glass stirring rod was used to mix the contents and to spread it up approximately 2½ inches on the side of vial. The corks used to stopper the vials were covered with a No. 39 souffle cup which was discarded after the test.

The use of Karo solution to wet the soil made it possible to keep flies alive on untreated soil for at least a week. Where preliminary tests were being run this was important since flies exposed to soil wet with water die of starvation in a relatively short time. The spreading of the soil up on the sides of the vial exposed a much greater surface to the flies than could be done with strips of toweling or similar methods. The mixture was more uniformly spread and stayed moist for a longer time than when toweling was used. The greater effectiveness of this method is illustrated by the results given in table 1.

The dose-effect method of Litchfield & Wilcoxon (1949) was used where an exact measurement of small amounts of insecticide residue was desired. This method consists of adding to two soils serial dilutions of the insecticide for which analysis is being made. One of these is the "unknown" or the soil to be analyzed while the other is a check. The difference between these two samples at the 50% mortality level on curves drawn on logarithmic-probability paper gives the amount of insecticide residue in the sample. Since the highest dosage of added insecticide in the series is 16 times the smaller, this method is applicable only to unknown samples containing relatively small quantities of insecticide. Where the soils to be

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Table 1.—Effect of method of exposure of gamma isomer of BHC on toxicity to *Drosophila melanogaster* adults.

TYPE OF CONTAINER	EXPOSURE OF SOIL	PER CENT DEAD AFTER 24 HRS.	
		0.4 p.p.m.	0.8 p.p.m.
1″×6″ test tube	On 1″×5″ strip of toweling	0.0	2.5
20×100 mm. vial	In bottom of vial	5.0	50.0
30×100 mm. vial	Up 1 in. on sides of vial	25.0	90.0
30×100 mm. vial	2 in. on sides of vial	40.0	93.0
30×100 mm. vial	3 in. on sides of vial	62.5	95.0

analyzed contained too large a residue of insecticide to test properly with this method the soil was diluted with untreated soil before use.

The first step in analyzing a soil for insecticide residue was to expose flies to the soil without the addition of more insecticide. A standard containing small amounts of the test insecticide was set up for comparison. By making observations at intervals a rough estimate of the amount of insecticide residue in the soil could be made. If a more accurate determination of the quantity of insecticide present was desired this estimate indicated whether dilution of the soil was necessary. The approximate amount of dilution needed was also indicated.

Where the soil was spread up on the side of the vials an exact count of the flies in the vial could be made only by removing the flies from the vial. If less than one-half of the flies were dead this could be readily ascertained by the number of flies above the treated area of the vial. Where only a few flies remained alive in a vial they could be satisfactorily counted by shaking them down into the clear part of the inverted vial. Dead flies were often difficult to count since they often become stuck in the soil. This made it imperative that the number of flies originally introduced into the vial be accurately determined.

RESULTS.—The amount of insecticide residue remaining on the different plots, as well as the original amount applied, is shown in table 2.

In general larger residues were found in the Clarksville samples. The greatest difference between areas occurred in the DDT plots. The largest residues on a percentage basis were found in the BHC-treated soils. The smaller residues found in the plots to which lime was added indicate that this material may have been of some benefit in reducing BHC residues. Growth of peach trees set 2 years after the insecticides were applied was extremely poor on the BHC plots whether lime was added or not. The low residues occurring on the Parathion-treated plots is not surprising.

Table 2.—Insecticide residue, in parts per million, remaining in soil 4½ years after application.

	P.P.M. APPLIED	LOCATION	
		Nashville	Clarksville
Lead arsenate	125.0	—	—
Lead arsenate[a]	125.0	—	—
BHC	15.0	4.2	10.2
BHC[b]	15.0	3.0	4.8
Parathion	15.6	0.52	0.16
Dieldrin	15.6	1.8	6.0
DDT	62.5	1.6	16.0
Methoxychlor	125.0	—	—
Chlordane	65.0	4.3	4.0

[a] 500 lbs. per acre of zinc sulfate added to these plots.
[b] 1 ton of hydrated lime per acre added to determine its effect in breaking down BHC.

Sometimes in the analysis of the data the lines drawn on the logrithmic-probability paper showed heterogeneity as indicated by the (Chi)2 test. A number of these tests were repeated. Usually the second test gave satisfactory (Chi)2 tests. The results of some of the tests that were repeated are shown in table 3. The reasonably close agreement between the two tests in encouraging. Indications are that even where the curves fail to meet the (Chi)2 test for goodness of fit, a close approximation of the residue present is obtained. This is based on the assumption that the test where (Chi)2 indicated homogeneity gives an accurate measurement of the residue.

Table 3.—Comparison of results where analyses were repeated when (Chi)2 test indicated heterogeneity.

INSECTICIDE	LOCATION	P.P.M.	
		Homogeneous	Heterogeneous
BHC (g.i.)	Clarksville	10.2	11.0
BHC+lime	Clarksville	4.8	5.4
BHC (g.i.)	Nashville	3.0	3.2
Dieldrin	Nashville	1.6	1.8
DDT	Clarksville	16.0	15.6
Chlordane	Nashville	4.3	3.8

The method described herein for testing for insecticide residues in the soil is considered entirely satisfactory for five of the seven insecticides used but appears to be inadequate for the other two. Lead arsenate showed some toxicity to the flies, but was very slow in its action. The number of flies dead after 1 week of exposure to lead arsenate does not appear to be primarily a function of dosage. Also the quantities required to kill 50% of the flies is too great. In one test 1,000 p.p.m. lead arsenate resulted in only 37.5% mortality of the flies after 9 days. The situation seems to be somewhat analogous for methoxychlor although the dosage and time of exposure required to kill 50% are less.

Additional uses for the technique described herein appear possible. Preliminary tests on plant material were not entirely satisfactory, but show promise. A fertilizer-insecticide mixture was submitted by the manufacturer to determine whether it contained the desired quantity of aldrin. The fertilizer sample was finely ground and added to a sample of soil. After proper dilution the soil sample was analyzed for aldrin residue. Results obtained were considered satisfactory although no other types of analysis were available for comparison.

Perhaps the great advantage for the described method of using *Drosophila* adults for bioassay is its increased sensitivity. This is due primarily to the large area of material to which the flies are exposed. A second factor is that weakened flies become ensnared in the wet soil.

These flies are counted as dead. This eliminates the problem of determining whether a given individual should be considered dead or alive.

Where limited facilities and personnel are a problem the simplicity of the described method of rearing and using *Drosophila* for bioassay is advantageous. The flies were reared and the tests were set up in a small constant temperature box held at 80° F. Humidity was not controlled. The test vials were prepared in a separate building and no insecticides except as diluted for testing were taken into the basement where the constant temperature box was located. Equipment used for rearing and handling the flies was relatively inexpensive.

SUMMARY.—Considerable amounts of insecticide remained in soils taken from two peach growing areas in Arkansas 4½ years after application. The insecticides in descending order of residue on a percentage basis were: BHC, dieldrin, DDT, chlordane, and parathion. The analyses were made using adults of *Drosophila melanogaster* exposed to a large area of soil wetted with Karo solution plus serial dilution of insecticide.

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Control of the Clover Root Borer in Virginia[1]

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The clover root borer, *Hylastinus obscurus* (Marsh.), has long been recognized as a serious pest of red clover. Marshall *et al.* (1949) found that 40% of the red clover plants in second-year fields in New York were infested by this insect, with 1.5 borers per plant; and that in the third year nearly 100% of the plants were infested, with 3.8 borers per plant. They reported that BHC dust at the rate of 1.5 pounds per acre of the gamma isomer gave more consistent control than any other material tested. Gyrisco & Marshall (1950) reported good control with 1 pound per acre of parathion or of gamma isomer of BHC, and with 2 pounds per acre of dieldrin or aldrin, all applied as dusts. App & Everly (1950) found that good control could be obtained with BHC dust at the rate of 1.25 pounds per acre of the gamma isomer of BHC and with 5 pounds of chlordane. Gyrisco *et al.* (1954) obtained satisfactory control with 0.5 pound per acre aldrin, dieldrin, or heptachlor, but found 1 pound more consistently effective. They also obtained good control with 1 pound per acre of lindane, isodrin, chlordane, and gamma isomer of BHC. Dusts were more reliable than sprays. None of these investigators reported significant increases in hay yields following treatments, but Gyrisco *et al.* (1954) reported significant increases in the persistence of clover stands following control of the clover root borer.

In recent years there have been complaints that red clover, an important crop in Virginia, was not maintaining stands long enough to justify its use in rotations. Observations showed that many clover fields in the State were heavily infested by root insects, among which the clover root borer was the most important in many locations. Red clover is ordinarily seeded in stands of small grain in March, and usually the first cutting for hay is not made until the second summer following the seeding. The crop is attacked by the clover root borer only slightly, if at all, during its first or seeding year, but the infestation often becomes very heavy during the second year.

Surveys in Augusta County in 1953 and 1954 showed that from 40 to 98 % of the plants in second-year clover fields were infested, with an over-all average of 81% infested. The average number of borers per plant in the different fields ranged from 1 to 6.7, with an average of 3.5 for all fields examined. By the summer of the third year practically all the plants were infested, and most of those in the more heavily infested fields had died. Although diseases were also involved in the plant mortality, the clover root borer appeared to be the most important factor and it may have predisposed the plants to entry of diseases.

In 1954 and 1955, tests of insecticides against the clover root borer were made in Augusta and Montgomery Counties to evaluate: (1) the most effective insecticide; (2) proper timing of application; and (3) the comparative effectiveness of several methods of application.

METHODS AND MATERIALS.—Several insecticides were tested as granules, as sprays, or as dusts. The plots in the experiments contained 0.01 acre, but were of various shapes, depending upon the space available. All treatments were applied to 1-year-old stands of clover, except that the fall treatments were made on clover that had been seeded the previous spring. All treatments were made during the first half of April, except as otherwise noted. A randomized block arrangement of plots was used, with three replications in Montgomery County, and at least five replications in Augusta County.

The granulated lindane used in the Montogmery County experiments was formulated on attaclay carrying 2% of the toxicant. The granulated BHC used in the Augusta County experiments contained 2% of the gamma isomer, and was prepared by impregnating granulated tobacco with a BHC concentrate of 40% gamma isomer content. The heptachlor used at location 4 in Augusta County was formulated on attaclay carrying 2.5% of

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the toxicant. All other granulated materials used in these experiments were formulated on tobacco carrying 2% of the toxicant.

The granulated insecticides were applied with a lawn fertilizer spreader. In the Montgomery County Experiments the amount of insecticide for each plot was weighed and placed in the previously calibrated spreader, and the entire amount applied to the plot, doubling back when necessary. In the Augusta County experiments a weighed portion of insecticide was placed in the previously calibrated spreader, all plots scheduled to receive that treatment were treated, and the insecticide remaining in the spreader was weighed to determine the actual rate of application.

The spray treatment at location 1 in Augusta County was made with an orchard sprayer equipped with a horizontal boom and high-pressure nozzles. The pressure and truck speed were calibrated to deliver 75 gallons of spray per acre. All other spray treatments were made with a compressed-air sprayer. The dust treatments were applied with a plunger-type hand duster.

Stand counts were made soon after the spring treatments were applied on areas selected at random in each plot. Similar counts were made on the same areas again late in August or in September to determine the percentage of the plants that had survived the summer. The degree of infestation by the clover root borer was determined by digging 10 plants at random in each plot in August, dissecting them, and counting the clover root borers in each.

Hay samples were cut from a strip 30 inches wide and extending the entire length of each plot at the time of the second cutting at location 1, and at the time of the second and third cuttings at location 3 in Augusta County. These stands were in their second year. The green weight of each sample was recorded and a 2-pound portion was air-dried. From these two weights the yield of cured hay from each plot was calculated. Yield data were not taken at the time of the first cuttings because damage by the clover root borer is not apparent in the first crop.

RESULTS.—The results of these experiments are shown in the accompanying tables. At Christiansburg (Table 1) dieldrin, heptachlor, isodrin, and endrin at 1 pound per acre, and chlordane at 5 pounds, gave better control of the clover root borer than malathion or methoxychlor at 5 pounds per acre. The results with aldrin and lindane at 1 pound per acre were intermediate. The infestation in this field was light.

At Blacksburg (Table 1) a comparison was made of spray and granule treatments with four different toxicants. All treatments gave results which were highly significantly different from the check. In each case the granulated form of the insecticide was somewhat more effective than the spray, but the difference was not significant.

At location 1 in Augusta County (Table 2) granulated BHC, aldrin, and dieldrin gave excellent control of a heavy infestation of the clover root borer, Granulated malathion and BHC spray failed to give satisfactory control. The failure of the BHC spray was probably due to excessive wind at the time of application, as sprays of BHC gave much more satisfactory results in later experiments. The survival of clover plants was significantly higher on the plots sprayed with BHC than on those treated with granulated malathion, although the control of the root borer was poorer.

Table 1.—Results of treatments for the control of the clover root borer, Montgomery County, Virginia, 1954.

TOXICANT AND FORM	ACTUAL TOXICANT POUNDS PER ACRE	CLOVER ROOT BORERS PER 10 PLANTS
Christiansburg[a]		
Dieldrin	1.0	1.0
Heptachlor	1.0	1.0
Chlordane	5.0	1.35
Isodrin	1.0	1.35
Endrin	1.0	1.5
Aldrin	1.0	2.5
Lindane	1.0	2.85
Malathion	5.0	4.85
Toxaphene	5.0	6.15
Check, no treatment	—	9.0
L.S.D. at 5% level		2.7
Blacksburg[b]		
Heptachlor, granules	1.0	4.65
Heptachlor, spray	1.0	5.65
Dieldrin, granules	1.0	6.35
Dieldrin, spray	1.0	9.65
Aldrin, granules	1.0	8.35
Aldrin, spray	1.0	12.5
Lindane, granules	0.75	9.65
Lindane, spray	0.75	14.65
Untreated check	—	19.9

[a] All toxicants were in the form of granules in this experiment.
[b] Differences between check and each treatment are highly significant (P = <.01). There was no significant difference between methods of application.

At location 2 (Table 2) BHC and methoxychlor were each applied as granules, dust, and spray in October 1954. All the BHC treatments gave excellent control of the clover root borer the following year, and reduced sharply the damage caused by the clover root curculio, *Sitona hispidula* (F.). In addition the granuler and spray BHC treatments gave excellent control of the meadow spittlebug the following spring. BHC dust was less effective against the spittlebug. None of the fall treatments with methoxychlor at 2.5 pounds per acre gave satisfactory control of the clover root borer, or either of the other insects.

At location 3 (Table 2) the BHC granules, dust, and spray gave results which did not differ significantly among themselves, but the low rate of methoxychlor used in this experiment was not satisfactory. At location 4 the lower rates of BHC granules were less effective than the 1-pound rate, but the results with 0.8 pound per acre were not significantly different.

In two experiments the time of application of the insecticides was varied (Table 3). At location 5 in 1954 treatments were applied in April and May. All treatments were fairly effective, including granulated methoxychlor at 2.4 pounds per acre, and BHC dust applied May 12, two weeks after the peak of activity of adult clover root borers.

At location 6 (Table 3) granulated heptachlor and granulated BHC were applied October 22, 1954, and March 8 and April 12, 1955. Also BHC dust and spray were applied on the last date. All the BHC treatments

Table 2.—Results of treatments for the control of the clover root borer, Augusta County, Virginia.

Toxicant and Form	Actual Toxicant Pounds per Acre	Clover Root Borers per 10 Plants	Per Cent Plants Surviving in Fall
Location 1, 1954			
BHC, γ-isomer, granules	1.18	0.2	90
BHC, γ-isomer, granules[a]	1.18	0.4	88
Aldrin, granules	1.0	0.2	91
Dieldrin, granules	2.25	1.8	94
Malathion, granules	1.7	37.2	42
BHC, γ-isomer, spray	1.0	48.6	64
Check	—	57.2	42
L.S.D. at 5% level		6.8	7
Location 2, 1954–55[b]			
BHC, γ-isomer, granules	1.0	1.2	84
BHC, γ-isomer, dust	1.0	3.0	78
BHC, γ-isomer, spray	1.0	3.4	79
Methoxychlor, dust	2.5	10.0	67
Methoxychlor, spray	2.5	14.4	57
Methoxychlor, granules	2.5	17.2	56
Check	—	24.6	41
L.S.D. at 5% level		5.0	15
Location 3, 1955[c]			
BHC, γ-isomer, granules	1.12	0	89
BHC, γ-isomer, spray	1.0	0.4	77
BHC, γ-isomer, dust	1.0	1.4	76
Methoxychlor, granules	1.45	16.8	55
Methoxychlor, granules	1.03	29.2	32
Check	—	32.0	36
L.S.D. at 5% level		3.7	14
Location 4, 1955[d]			
BHC, γ-isomer, granules	1.0	2.0	70
Heptachlor, granules	1.5	4.8	70
BHC, γ-isomer, spray	1.0	5.8	67
BHC, γ-isomer, granules	0.8	7.8	60
BHC, γ-isomer, dust	1.0	12.4	56
BHC, γ-isomer, granules	0.6	14.2	45
Methoxychlor, granules	1.25	28.2	34
Check	—	33.2	22
L.S.D. at 5% level		6.8	15

[a] This treatment was applied April 19. All other treatments in this experiment were applied on April 2 and 3.
[b] All treatments applied October 8, 1954. Infestation and stand counts were made in 1955.
[c] Treated April 6, 1955.
[d] Treated April 5, 1955.

gave good control of the borer, as did the March application of heptachlor. The October and April treatments with heptachlor gave poorer results than most of the other treatments.

The yield data obtained in two experiments are not presented in the tables. At location 1 samples of hay were taken at the time of the second cutting in the second year of growth. The yields in the plots treated with granulated BHC, granulated aldrin, or granulated dieldrin were highly significantly different from those on the check plots or those treated with BHC spray or granulated malathion. The correlation between the degree of control of the clover root borer and the yield was fairly close. At location 3 samples were taken from each plot

Table 3.—Results of treatments for the control of the clover root borer, varying the time of application, Augusta County, Virginia.

Toxicant and Form	Actual Toxicant Pounds per Acre	Date Applied	Clover Root Borers per 10 Plants	Per Cent Plants Surviving in Fall
Location 5, 1954				
BHC, γ-isomer, granules	1.2	April 29	2.2	77
Methoxychlor, granules	2.4	April 29	3.0	72
BHC, γ-isomer, dust	1.0	April 15	3.2	70
BHC, γ-isomer, granules	1.2	April 15	5.0	69
BHC, γ-isomer, dust	1.0	April 29	5.6	65
BHC, γ-isomer, dust	1.0	May 12	7.4	53
Check	—	—	30.2	35
L.S.D. at 5% level			6.6	10.3
Location 6, 1954–55				
Heptachlor, granules	1.0	October 22, 1954	7.6	61
BHC, γ-isomer, granules	1.0	October 22, 1954	1.8	79
Heptachlor, granules	1.0	March 8, 1955	0.2	80
BHC, γ-isomer, granules	1.0	March 8, 1955	3.8	66
Heptachlor, granules	1.0	April 12, 1955	10.0	63
BHC, γ-isomer, granules	1.0	April 12, 1955	3.2	79
BHC, γ-isomer, spray	1.0	April 12, 1955	1.8	79
BHC, γ-isomer, dust	1.0	April 12, 1955	2.2	68
Check	—	—	34.8	35
L.S.D. at 5% level			4.0	12.4

at the time of the second and third cuttings. The yields on the plots treated with BHC dust, spray, and granules were significantly higher than yields on the check plots or those treated with 1 pound per acre of methoxychlor. Again there was a close correlation between the degree of control of the clover root borer and the yield. The differences in yield were largely due to differences in survival of plants, but there was also a noticeable decrease in the vigor and growth of the surviving plants where the clover root borer was not controlled.

SUMMARY.—Red clover, an important crop in Virginia, has been found to be heavily infested with clover root borer, *Hylastinus obscurus* (Marsh.), in parts of the state. Applications of BHC at the rate of 1 pound of gamma isomer per acre in dusts, sprays, and granulated formulations resulted in good control of this insect. Other insecticides such as dieldrin, aldrin, isodrin, endrin and heptachlor at 1 pound of actual toxicant per acre, and chlordane at 5 pounds of actual toxicant per acre, also proved to be effective in the control of the clover root borer. Neither methoxychlor, malathion nor toxaphene at 2.5, 5.0, and 5.0 pounds per acre, respectively, was as effective in controlling the borer. Variation in the time of application showed that control was obtained with gamma isomer of BHC in fall, winter or spring applications. On the other hand, applications of heptachlor made at the same times indicated that winter treatment was somewhat better than fall or spring treatments. Yields from treated plots showed a close correlation with the degree of control of the clover root borer.

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Effectiveness of Cyclethrin with Various Synergists Against Body Lice[1]

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Various chemicals have been found to be effective synergists for pyrethrins and allethrin against the body louse, *Pediculus humanus humanus* L. (Bushland *et al.* 1944a, Carson & Eddy 1949, and Eddy *et al.* 1954). Recently some of these chemicals and others have been subjected to similar tests for synergism with the new synthetic pyrethrins-type insecticide known as cyclethrin (Haynes *et al.* 1954). These materials were evaluated both as cloth impregnants in beaker tests as described by Bushland *et al.* (1944b), and as powders in patch tests as described by Eddy (1952). A 1:10 ratio of toxicant to synergist was used in all tests. Pyrethrins and allethrin in combination with sulfoxide were tested as standards of comparison. The body lice were from a laboratory colony maintained on domestic rabbits.

BEAKER TESTS.—In the beaker tests the materials were dissolved in acetone and 0.7 ml. of the solution was applied by pipette to circular pieces of woolen cloth 1.5 inches in diameter. The treated pieces were suspended on pins, allowed to dry for about an hour, and then placed in a 50-ml. beaker with 10 adult lice of each sex. The beakers were kept at 28° C. and 60 to 70% relative humidity. Mortality records were taken after 24 hours. The treated cloths that were effective in the initial tests were retested at various intervals until they no longer caused satisfactory mortality of lice.

The results of tests with cyclethrin at a concentration of 0.005% alone and in combination with 0.05% of the various synergists are presented in table 1. The *alpha*-

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[2] D. L. Van Natta assisted in conducting some of the tests, and M. D. Couch, P. N. Mason, Jr., and R. B. Bonner reared the lice.

Table 1.—Residual effectiveness of cyclethrin, alone and in combination with selected synergists, in beaker tests against body lice.

SYNERGIST	ORLANDO NO.[a]	PER CENT MORTALITY AFTER—						
		Initial	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Acetic acid, phenyl-, *alpha*-isopropylpiperonyl ester	20616	100	100	90	85	75	50	—
Benzene, 1,2-methylenedioxy-4-[2-(octylsulfonyl)propyl]-	18323	100	95	95	85	85	25	—
Chrysanthemumic acid,								
alpha-allylpiperonyl ester	20003	100	100	100	100	90	85	75
alpha-cyclohexylpiperonyl ester	20426	100	100	85	85	90	65	35
alpha-isopropylpiperonyl ester	20337	100	100	100	100	100	100	90[b]
4-(3,4-methylenedioxyphenyl)-*sec*-butyl ester	20244	100	100	100	100	100	100	85[c]
alpha-(3-phenylpropyl)piperonyl ester	20425	100	100	65	45	—	—	—
alpha-propylpiperonyl ester	20091	100	100	100	100	100	85	55
6-propylpiperonyl ester	20687	100	95	70	75	70	35	—
Fencholic acid, *alpha*-allypiperonyl ester	20332	100	100	100	100	100	95	70
N-Isobutylundecenamide (IN-930)	546	65	15	—	—	—	—	—
MGK 264	8184	100	100	95	100	100	35	—
Piperonyl butoxide	14250	100	100	95	75	85	40	—
Propyl isome	15266	100	95	80	65	35	—	—
Sulfoxide	16634	100	95	70	75	75	70	5
Standards:								
Pyrethrins+sulfoxide		100	100	100	100	100	100	100[d]
Allethrin+sulfoxide		100	100	90	100	95	45	—
No synergist:								
Cyclethrin		5	—	—	—	—	—	—
Pyrethrins		20	—	—	—	—	—	—
Allethrin		10	—	—	—	—	—	—
Untreated		5	0	0	0	0	5	0

[a] The synergists in the 20000 series were synthesized by W. F. Barthel.
[b] 90% after 49 days, 55% after 56 days.
[c] 65% after 49 days.
[d] 95% after 56 days.

Table 2.—Effectiveness of cyclethrin, pyrethrins, and allethrin at different concentrations, alone and with sulfoxide as a synergist, in beaker tests.

Toxicant	Per Cent Mortality at Indicated Concentration of Toxicant[a]			
	0.005%	0.0025%	0.001%	0.0005%
Alone:				
Pyrethrins	20	10	—	—
Allethrin	40	10	—	—
Cyclethrin	0	—	—	—
With sulfoxide:				
Pyrethrins	100	100	40	25
Allethrin	100	100	65	10
Cyclethrin	100	75	40	15

[a] Mortality in check lots ranged from 0 to 10%.

allylpiperonyl (20003), *alpha*-propylpiperonyl (20091), 4-(3,4-methylenedioxyphenyl)-*sec*-butyl (20244), and *alpha*-isopropyl piperonyl (20337) esters of chrysanthemumic acid and the *alpha*-allylpiperonyl ester of fencholic acid (20332) were the most effective synergists, each combination giving at least 85% knockdown and kill through the 35th day after treatment. Many of the materials were superior to sulfoxide (16634); however, pyrethrins with sulfoxide was more effective than cyclethrin with any synergist, and allethrin with sulfoxide was slightly less effective than cyclethrin with some of its better synergists. None of the synergists alone were toxic to lice at the concentration used.

Table 2 shows the results of additional tests with cyclethrin, pyrethrins, and allethrin, alone and in combination with sulfoxide, to determine the minimum effective concentrations. Cyclethrin was slightly less effective than either pyrethrins or allethrin, when used alone or in combination with sulfoxide.

Patch Tests.—In the patch tests swatches of balbriggan cloth 4.9 inches square were treated with 0.5 gram of pyrophyllite powder impregnated with the insecticides. To facilitate handling, the treated cloths were fastened to slightly larger plywood panels. Metal rings from wide-mouthed fruit-jar lids, held in position with rubber bands, were used to confine the lice on the treated cloths. The testing procedures were the same as for the beaker tests.

Table 3 shows the results of tests of cyclethrin, pyrethrins, and allethrin in pyrophyllite powders at a concentration of 0.1%, alone and in combination with sulfoxide.

Cyclethrin was slightly slower in knockdown than allethrin or pyrethrins when each was combined with sulfoxide. Synergized allethrin and cyclethrin were effective for 14 days, and synergized pyrethrins for 16 days.

Summary.—Laboratory tests were conducted with 15 synergists in combination with cyclethrin against the body louse, *Pediculus humanus humanus* L., in comparison with pyrethrins and allethrin standards. The mixtures were tested as cloth impregnants in beakers and as powders applied to small cloth patches.

In the beaker tests the *alpha*-allylpiperonyl, the *alpha*-propylpiperonyl, the 4-(3,4-methylenedioxyphenyl)-*sec*-butyl, and the *alpha*-isopropylpiperonyl esters of chrysanthemumic acid, and the *alpha*-allylpiperonyl ester of fencholic acid were the most effective synergists for cyclethrin. However, pyrethrins with sulfoxide was more effective than cyclethrin with any synergist, and allethrin with sulfoxide was slightly less effective than cyclethrin with some of its better synergists.

Pyrethrins and allethrin were completely effective at a lower concentration than cyclethrin, when each was used in combination with sulfoxide.

In pyrophyllite powders cyclethrin was slightly slower in knockdown than allethrin or pyrethrins when combined with sulfoxide. On a residual basis synergized cyclethrin and allethrin were less effective than pyrethrins.

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Table 3.—Residual effectiveness of powders containing cyclethrin, pyrethrins, and allethrin, alone and in combination with sulfoxide, as a synergist.

Toxicant	Per Cent Knockdown in—			Per Cent Mortality After—					
	30 Minutes	1 Hour	3 Hours	Initial	3 Days	7 Days	14 Days	16 Days	21 Days
Alone:									
Pyrethrins	40	40	40	25	—	—	—	—	—
Allethrin	0	0	0	5	—	—	—	—	—
Cyclethrin	0	0	0	0	—	—	—	—	—
With sulfoxide[a]:									
Pyrethrins	100	100	100	100	100	100	100	90	5
Allethrin	50	50	95	100	100	95	90	10	—
Cyclethrin	25	25	100	100	100	100	90	30	—
Checks									
Pyrophyllite	0	0	0	0	0	0	5	5	0
Untreated	0	0	0	0	0	0	0	10	5

[a] 1% of sulfoxide alone gave no mortality.

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Chemical Structure and Insecticidal Activity of a Number of Systemic Insecticides[1]

CARL JOHANSEN[2]

A number of investigators have attempted to show general correlations between chemical structure and insecticidal activity in phosphate systemics. Schrader (1946) utilized several such apparent correlations in developing new phosphate compounds. In work with some fluorophosphates, he noted that those containing the C—P linkage were highly toxic to animals but not more insecticidal than those containing the C—O—P linkage; and that the P—N linkage reduced toxicity to warm-blooded animals.

Considering phosphate, pyrophosphate, and thiophosphate insecticides, Schrader (1947) reported that the active groups appeared to be $(RO)_2P(X)—O—$ and $(R_2N)_2P(X)—O—$, where X equals either O or S. He found that the P=S (thiophosphate) esters tended to be less toxic to animals, but not less insecticidal than the corresponding P=O (phosphate) esters. However, Schrader stated that replacement of the phosphoryl group with thiophosphoryl reduced the systemic effectiveness of phosphates. Several workers have shown this to be true in comparing parathion and paraoxon (e.g. Metcalf & Carlson 1950).

Kilby (1949) and Martin (1949, 1951) reiterated some of Schrader's principles and cited data of their own and of others concerning these ideas. The chemical group which was common to all the active compounds studied by Chamberlain & Hoskins (1951) also was similar to Schrader's "toxophore."

Ludvick & Decker (1947) found that simple organic phosphorus compounds were inferior to nicotine in toxicity to aphids, whereas polyphosphate derivatives were more highly toxic than nicotine.

In the present study several series of thiophosphate and pyrophosphate compounds were obtained for comparisons of structural changes and insecticidal activity by both contact and systemic action. The diethyl thiophosphoric acid esters[3] of alcohols and seleno alcohols of sulfides with varying chain lengths, were arranged into three logical groups of four materials. Group I was designed to test the effectiveness of oxygen vs. selenium ester linkages; Group II, of changes in the group between the ester and the sulfur linkage; and Group III, of changes in the end group beyond the sulfur linkage. Demeton[4] was included in each group as a standard for comparisons between groups. For convenience, these groups will be referred to as series I, II, and III (table 1). All of these compounds were formulated with emulsifier 8139[5] so that the weight-volume relationship was equal to that of the commercial 32.1% demeton formulation. Two of the compounds, 21/125 and E-1495, required a greater proportion of the emulsifier in order to be completely solubilized when diluted with water.

One group of pyrophosphate materials[6] was also tested for both contact and systemic action (series IV). These compounds represent the complete series which may be obtained by stepwise replacement of the ethoxy groups of TEPP with dimethylamido groups. Schrader (1951) discussed the six materials thus formed and outlined the reactions used in their synthesis. He also gave comparative data obtained from insecticidal screening of the compounds. Since all of these materials were soluble in water, no other solvents were necessary in formulating them for testing. The pyrophosphates are listed in table 2.

METHODS AND MATERIALS.—*Contact Tests.*—Chrysanthemum aphid, *Macrosiphum sanborni* (Gill.), was employed as the test insect for contact action experiments. Aphids were collected from culture plants kept in a 60° F. (night temperature) greenhouse and sprayed with the phosphate compounds by means of an endless-belt type laboratory spray apparatus similar to that described by Siegler & Munger (1935). The sprayer was a Spraying Systems Co., Chicago, Model JH 2850 with a number 70 disc. An air pressure of 50 mm. of mercury was used for all tests and the height of the sprayer orifice above the surface being sprayed was 47.5 cm. This sprayer applied 0.6 mg. of spray mixture per sq. cm. under these conditions. The aphids were placed in stacking bowls on the conveyer belt and were allowed to pass through the spray cone once.

[1] Special thanks are due to the late L. B. Norton whose suggestions and criticisms were most helpful in carrying out the work reported. This is a part of the investigations conducted at Cornell University, 1949–1951, under the direction of the late W. E. Blauvelt. Accepted for publication February 28, 1956.

[2] Now Assistant Entomologist at State College of Washington, Pullman.

[3] All of the thiophosphate samples were approximately 90% technical grade (supplied by Farbenfabriken Bayer Co., Germany); most of these materials are covered by U. S. Patent number 2,571,989.

[4] Heat isomerization similar to that which occurs with parathion is also a property of demeton and presumably all of the other thiophosphates (Kosolapoff 1950). Therefore, all formulations probably contain a mixture of two types of compounds.

[5] "Thiosolve," polyethylene glycol ether, Geary Chemical Corp.

[6] TEPP technical 40%, "Nifos T," Monsanto Chemical Co.; M2/34 technical 89.8% 15/8B technical 90%, 15/8 technical 90%, and M2/35 technical 93.6% supplied by Farbenfabriken Bayer Co., Germany; Schradan, octamethyl pyrophosphoramide technical 70%, Monsanto Chemical Company.

Table 1.—Thiophosphate Compounds.

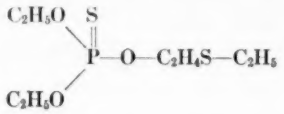
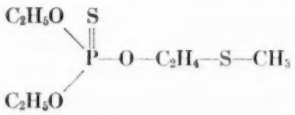
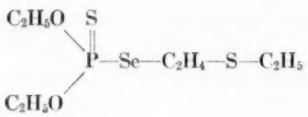
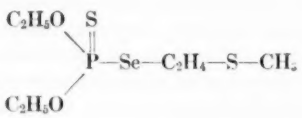
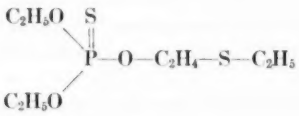
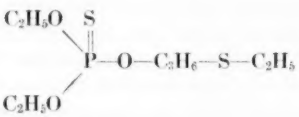
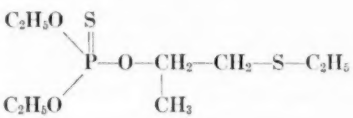
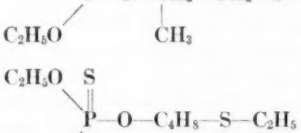
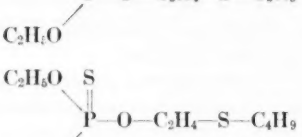
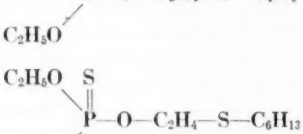
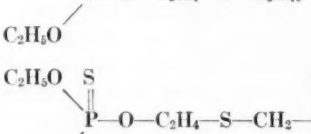
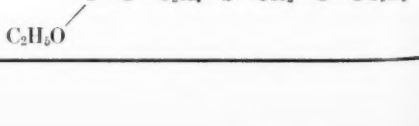
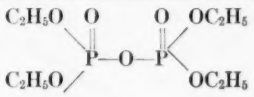
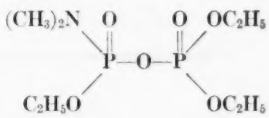
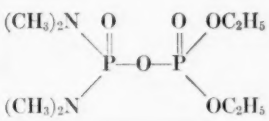
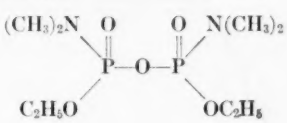
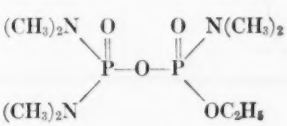
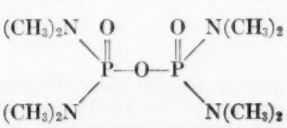
Schrader's Code No.	Chemical Name	Structural Formula
	Series I	
E-1059 (demeton)	O,O-diethyl-O-ethylmercaptoethyl thiophosphate	
21/83	0,0-diethyl-0-methylmercaptoethyl thiophosphate	
20/58	0,0-diethyl-Se-ethylmercaptoethyl seleno thiophosphate	
20/86	0,0-diethyl-Se-methylmercaptoethyl seleno thiophosphate	
	Series II	
E-1059 (demeton)	0,0-diethyl-0-ethylmercaptoethyl thiophosphate	
21/125	0,0-diethyl-0-ethylmercapto-n-propyl thiophosphate	
E-1486	0,0-diethyl-0-ethylmercaptoisopropyl thiophosphate	
E-1484	0,0-diethyl-0-ethylmercapto-n-butyl thiophosphate	
	Series III	
E-1059 (demeton)	0,0-diethyl-0-ethylmercaptoethyl thiophosphate	
E-1492	0,0-diethyl-0-n-butylmercaptoethyl thiophosphate	
E-1495	0,0-diethyl-0-n-hexylmercaptoethyl thiophosphate	
E-1531	0,0-diethyl-0-carbethoxymethyl-mercaptoethyl thiophosphate	

Table 2.—Pyrophosphate Compounds.

SCHRADER'S CODE NO.	CHEMICAL NAME	STRUCTURAL FORMULA
(TEPP)	tetraethyl pyrophosphate	
M2/34	dimethyl amido triethyl pyrophosphate	
15/8B	asymmetrical bis (dimethylamido) diethyl pyrophosphate	
15/8	symmetrical bis (dimethylamido) diethyl pyrophosphate	
M2/35	tris (dimethylamido) monoethyl pyrophosphate	
13/163 (schradan)	bis (bis dimethylamido) pyrophosphate	

Treated aphids were placed in 10-cm. petri dishes which had been fitted with a 9-cm. filter paper in each half. The top filter paper was moistened with one ml. of distilled water which caused it to adhere to the dish, and to maintain a high humidity during the test; and a fresh leaf from an untreated chrysanthemum plant was placed in the bottom. These cages were placed in a 75° F. ± 1° holding cabinet and mortality counts of the adult aphids were taken after 24 hours.

Systemic Tests.—An apparatus was constructed for the systemic tests to eliminate the effect of direct fumigant action. A 20-foot rectangular air-duct of masonite with 1″×2″ bracing was attached to a 60 cycle, 1/250 h.p. furnace blower so that an air column would be drawn through the duct. Metal drain pipe was attached to the blower outlet in order to conduct fumes out of the greenhouse in which the apparatus was used. The top of the duct was slotted at intervals and one side was hinged as a series of flaps. This allowed the introduction of potted chrysanthemum plants with the foliage protruding through the slots and the pots enclosed in the ventilated duct. Plants of the variety Indianapolis Yellow[7] in 4-inch pots were used for the tests. Air movement in the duct was sufficient to draw air through the slots when the blower was on. As an added precaution against fumes from the surface of the soil, pot labels were placed over the slots (Figure 1).

Adult chrysanthemum aphids were transferred to the plants, and water mixtures of the test materials were then poured into the pots by means of funnels through the top of the duct. Counts of adult aphids were taken 6 days after treatments were applied.

DISCUSSION.—Data obtained in these tests were plotted as log dose against probit mortality. The Bliss method of probit analysis was utilized to calculate straight-line plots for each curve and to establish fiducial limits at the 5% level (Bliss 1935).

Results of contact tests of the thiophosphate series are shown in table 3 and graphically in figures 2, 3, and 4. The plots in figure 2 indicate that the selenium-containing compounds tend to be more effective than the non-selenium compounds, and that within each of these subgroups the ethyl forms are more active by contact than the methyl forms. Data presented in figure 3 show that increases above ethyl in the length of the variable alkyl chain between the sulfur and the thiophosphate nucleus decrease the contact effectiveness. Similarly, results presented in figure 4 indicate that increases above ethyl at the free end of the variable alkyl chain also decrease the contact effectiveness.

These general tendencies resemble those of most other workers. Metcalf & March (1949) found that certain methyl and ethyl phosphate esters were more insecticidally active than higher homologs. Woodcock & Stringer (1951) reported that increasing the size of the groups at-

[7] Yoder Brothers Inc., Barberton, Ohio, supplied 100 rooted cuttings of Indianapolis Yellow every month for the duration of the tests.

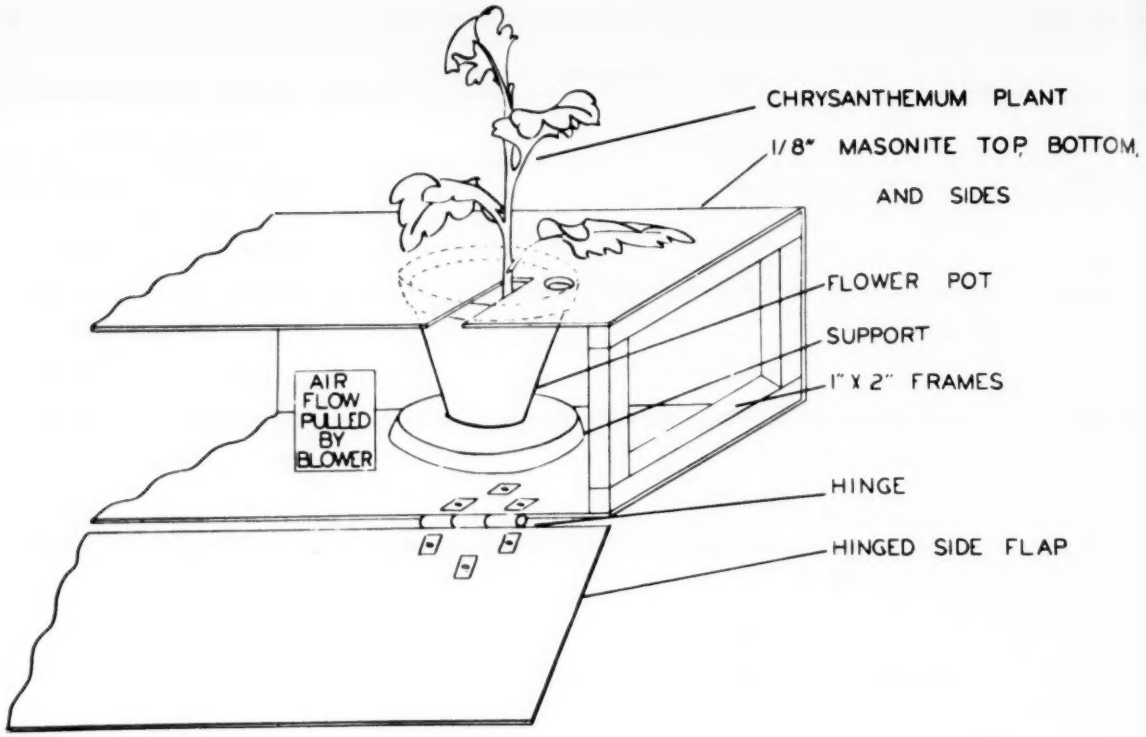


FIG. 1.—Apparatus used in testing systemic action.

Table 3.—Insecticidal Effectiveness of Thiophosphates Compared at SD_{50} for Chrysanthemum Aphid.

	CONTACT ACTION		SYSTEMIC ACTION	
COMPOUND	LD_{50} in Per Cent Technical	5% Fiducial Limits at LD_{50}	LD_{50} in Lb. Tech. per 1000 Sq. Ft.	5% Fiducial Limits at LD_{50}
		0.001		0.001
20/58	0.002		0.003	
		.004		.008
		.002		.001
20/86	.003		.001	
		.006		.002
		.004		.003
Demeton[a]	.006		.007	
		.008		.013
		.004		.002
21/83	.007		.005	
		.011		.010
		.005		.017
E-1486	.009		.023	
		.016		.032
		.001		.058
E-1484	.012		.105	
		.204		.174
		.009		.032
E-1492	.015		.065	
		.027		.115
		.010		.245
21/125	.020		.302	
		.055		.363
		.015		.047
E-1495	.021		.098	
		.029		.251
		.123		1.15
E-1531	.214		1.95	
		.372		3.89

[a] Average of three standards.

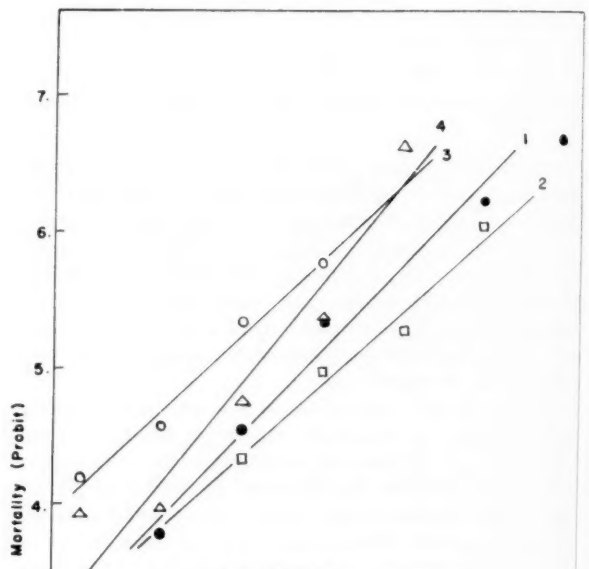


FIG. 2.—Comparison of Series I materials (Thiophosphates) against chrysanthemum aphid based on contact action.

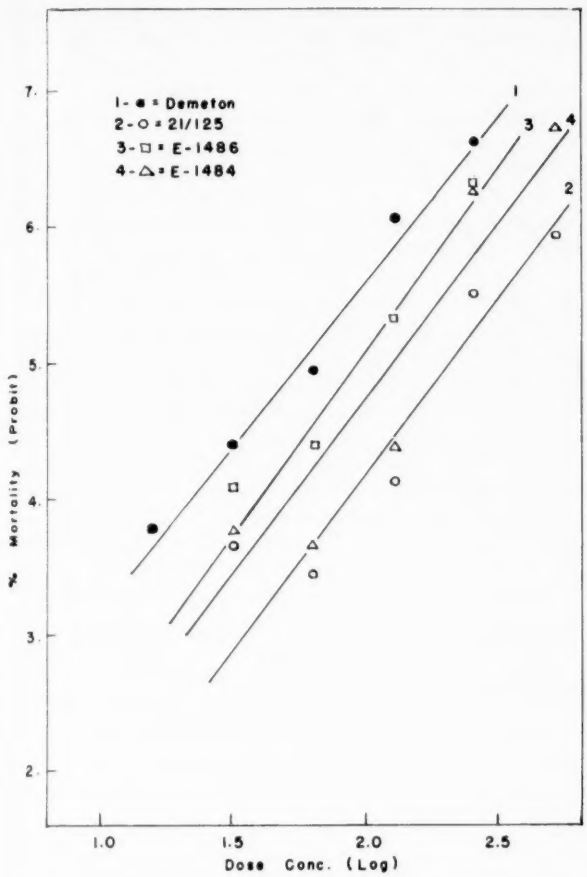


FIG. 3.—Comparison of Series II materials (Thiophosphates) against chrysanthemum aphid based on contact action.

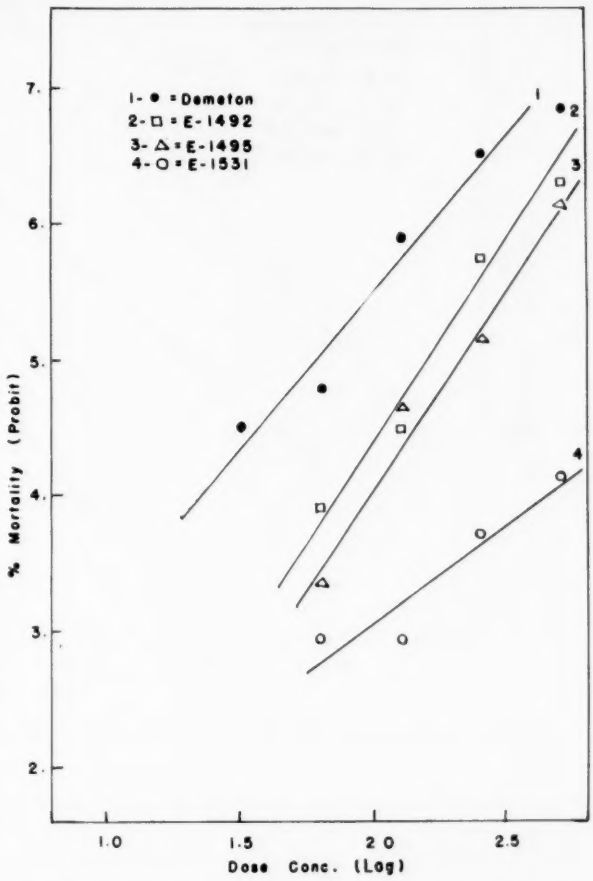


FIG. 4.—Comparison of Series III materials (Thiophosphates) against chrysanthemum aphid based on contact action.

tached to the central phosphorus atom caused a reduction of insecticidal activity for the series of phosphate compounds they examined. Ludvick & Decker (1947) showed that increasing the length of one carbon chain tends to increase the toxicity of the phosphate ester nucleus up to a certain point, then to decrease it.

An interesting point is that the ethylmercaptoisopropyl form, E-1486, is much closer in effectiveness to the series I materials than n-propyl or higher homologs. This agrees with the findings of Ludvick & Decker (1951) who noted that isopropyl alone of the higher alkyl groups approached the ethyl group in maintaining the toxicity of certain phosphates. Since both groups have a main carbon chain of the same length, it appears that the similarity in activity may be due to a similarity in spatial configuration.

Contact tests of the pyrophosphate group (figure 5 and table 4) showed that the contact toxicity decreased markedly as the ester groups were replaced by dimethylamido groups. Schrader (1951) stated that he had developed the amido compounds in an effort to avoid the rapid hydrolysis of TEPP.

The rate of hydrolysis, like the contact activity, decreases with increasing substitution of amido for ester groups. It is not possible from the present data to tell whether the speed of hydrolysis is directly connected with the insecticidal activity, or whether the two are

Table 4.—Insecticidal Effectiveness of Pyrophosphates Compared at SD for Chrysanthemum Aphid.

	Contact Action		Systemic Action	
Compound	LD_{50} in Per Cent[a]	5% Fiducial Limits at LD_{50}	LD_{50} in Lb.[a] per 1000 Sq. Ft.	5% Fiducial Limits at LD_{50}
		0.005		1.00
TEPP	0.007		7.94	
		.010		63.1
		.019		.013
M2/34	.025		.022	
		.033		.033
		.023		.015
15/8B	.058		.038	
		.115		.076
		.082		.060
15/8	.090		.063	
		.099		.066
		.219		.066
M2/35	.302		.096	
		.407		.145
		.389		.081
Schradan	.550[b]		.129	
		.776		.204

[a] Based on actual schradan or TEPP and technical M2/35, 15/8, 15/8B, or M2/34.

[b] By interpolation between the two adjacent ends of the "broken" curve.

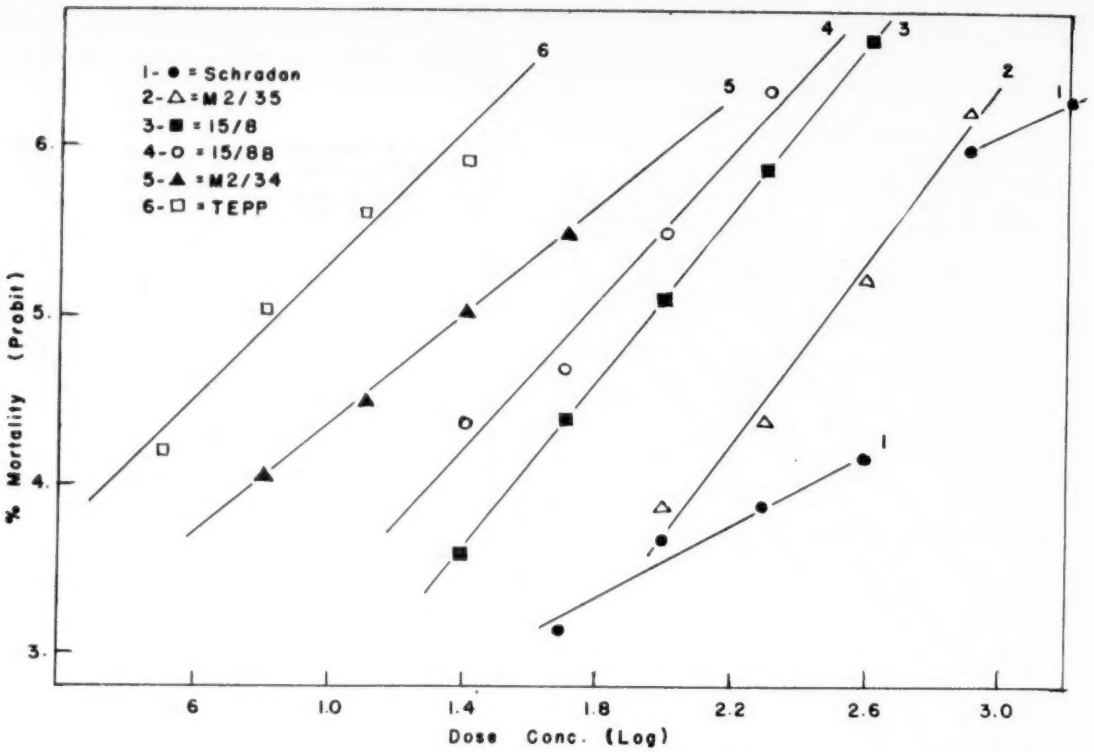


FIG. 5.—Comparison of Series IV materials (Pyrophosphates) against chrysanthemum aphid based on contact action.

independent results of the structural changes. Topley (1950) suggested that possibly the mode of action of phosphate insecticides involves the phosphorylation of enzyme protein, and if the phosphate is so inert that it does not attack the hydroxyl bonds of water, the inertness might also apply to phosphorylation of other substrates. He reasoned that the hydrolytic stability of schradan might be associated with the absence of enzyme inhibiting power *in vitro* and the absence of physiological action.

Metcalf & March (1949) and Sexton (1950) noted that the substitution of dimethylamido groups for ethoxy groups on phosphate insecticide molecules reduced the contact activity. Their statements are obviously in accord with the results obtained for the six compounds of series IV.

The hydrolysis of the first three compounds of table 2 was rapid enough to require special care in the experimental work. Table 5 shows how rapidly their insecticidal activity was reduced on standing in water solution. This effect was minimized in the regular insecticidal tests by applying all materials of the series immediately after dilution. In contrast with these esters, comparative tests against chrysanthemum aphid with 1:1000 water solu-

Table 5.—Effect of Hydrolysis upon the Activity of Several Pyrophosphates against Chrysanthemum Aphid.

Material	Per Cent Concentration	Contact: Per Cent Mortality from Fresh Solution	Contact: Per Cent Mortality from 6-Hour Solution	Lb. per 1000 Sq. Ft.	Systemic: Per Cent Mortality from Fresh Solution	Systemic: Per Cent Mortality from 6-Hour Solution
15/8B	0.1	51	11	0.25	76	4
M2/34	.05	97	65	0.25	75	1
TEPP	.025	86	4	2.0	11	0

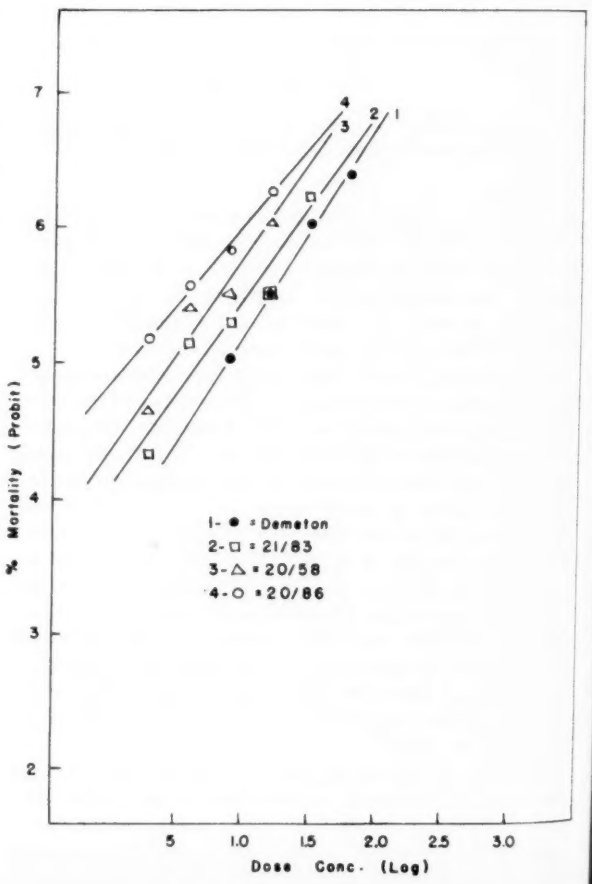


FIG. 6.—Comparison of Series I materials (Thiophosphates) against chrysanthemum aphid based on systemic action.

tions of schradan and 15/8 which had been held in stoppered flasks for 18 months, with fresh 1:1000 solutions, revealed no significant differences in mortalities.

Reference to figure 5 indicates an interesting phenomenon exhibited by the contact dosage-mortality curve for schradan. The plotted data form a curve with two separate parts, which effect was probably caused by impurities in the schradan sample.

The LD_{50}s and their 5% fiducial limits listed in table 4 show that most of the differences between the curves are significant at LD_{50}.

Data from the systemic experiments with the thiophosphate series are presented graphically in figures 6, 7, and 8. The curves for series I reveal that the selenium-containing compounds are more effective than the non-selenium compounds. However, the methyl forms tend to be more effective than the ethyl forms in each subgroup, which is the reverse of the findings for contact action. The greater effectiveness of the methyl compounds in fumigation from the foliage of soil-treated plants (confirmed in other studies) may add to their systemic insecticidal activity. The results presented for series II and III again show that increases above ethyl on either end of the variable alkyl chain decrease the effectiveness. The sequence within each of these two groups is the same as that for contact action. The sequence between groups is slightly different, however, the effect of the end group being less pronounced and that of the intermediate group more pronounced in systemic than in contact effect. Since the effect of the length of the carbon chain may logically be upon penetration, it appears that penetration into the insect and into the plant are affected somewhat similarly in this case.

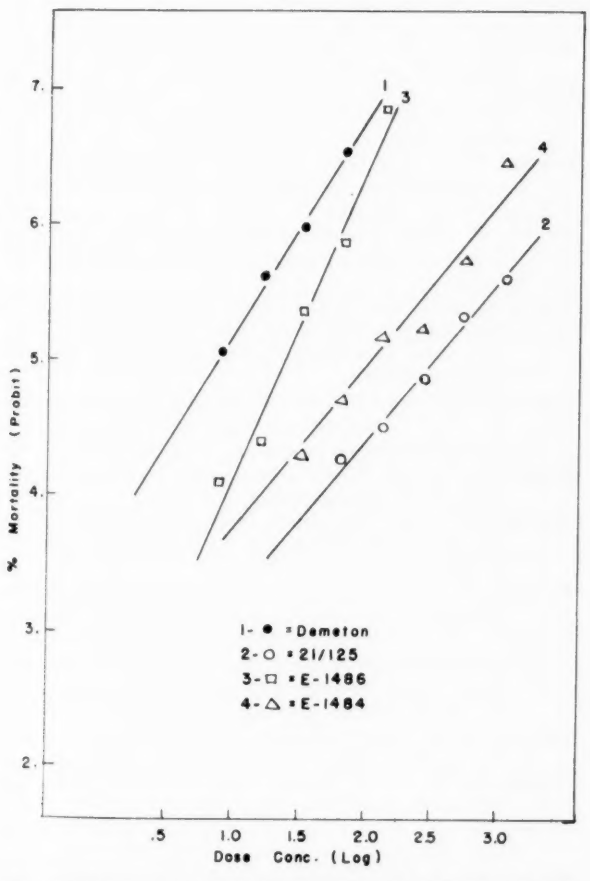


FIG. 7.—Comparison of Series II materials (Thiophosphates) against chrysanthemum aphid based on systemic action.

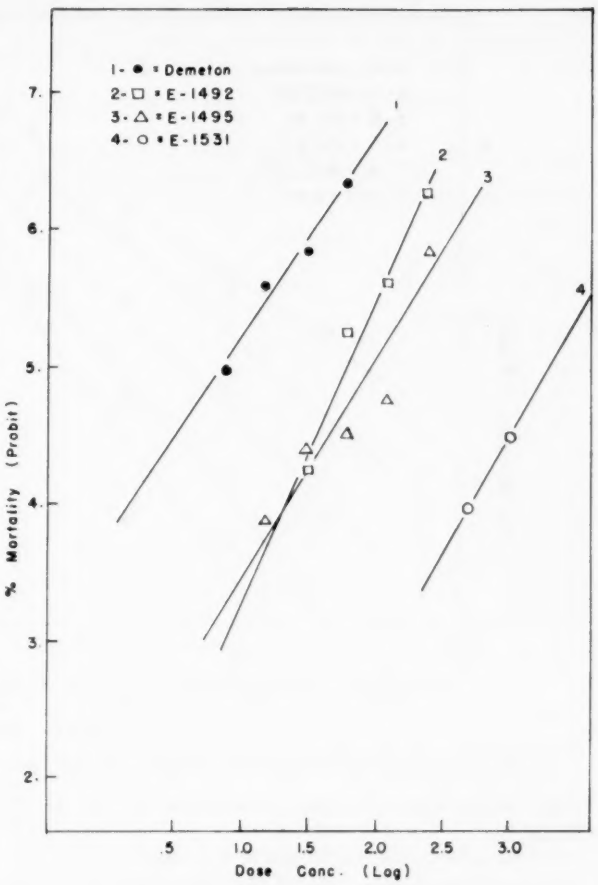


FIG. 8.—Comparison of Series III materials (Thiophosphates) against chrysanthemum aphid based on systemic action.

Results of the systemic action tests for series IV are shown in figure 9. Five of the materials appear in the same sequence as for contact action, but they are much closer together, showing fewer significant differences. Hydrolysis could be expected to reduce the inherent effectiveness of the less stable compounds more in the systemic than in the contact tests, accounting at least partially for the closer grouping. TEPP is apparently far below the others.

CONCLUSIONS.—Both contact and systemic insecticidal tests against the chrysanthemum aphid were conducted with two series of organic phosphorus compounds. One series contained diethyl thiophosphoric acid esters of alcohols and seleno alcohols of sulfides with varying chain lengths. The other contained TEPP and related pyrophosphates which had varying substitutions of ethoxy by dimethylamido groups.

In the thiophosphate series, the following points were noted:

1. Compounds containing a P—Se—C linkage in one group appeared more effective both by contact and sys-

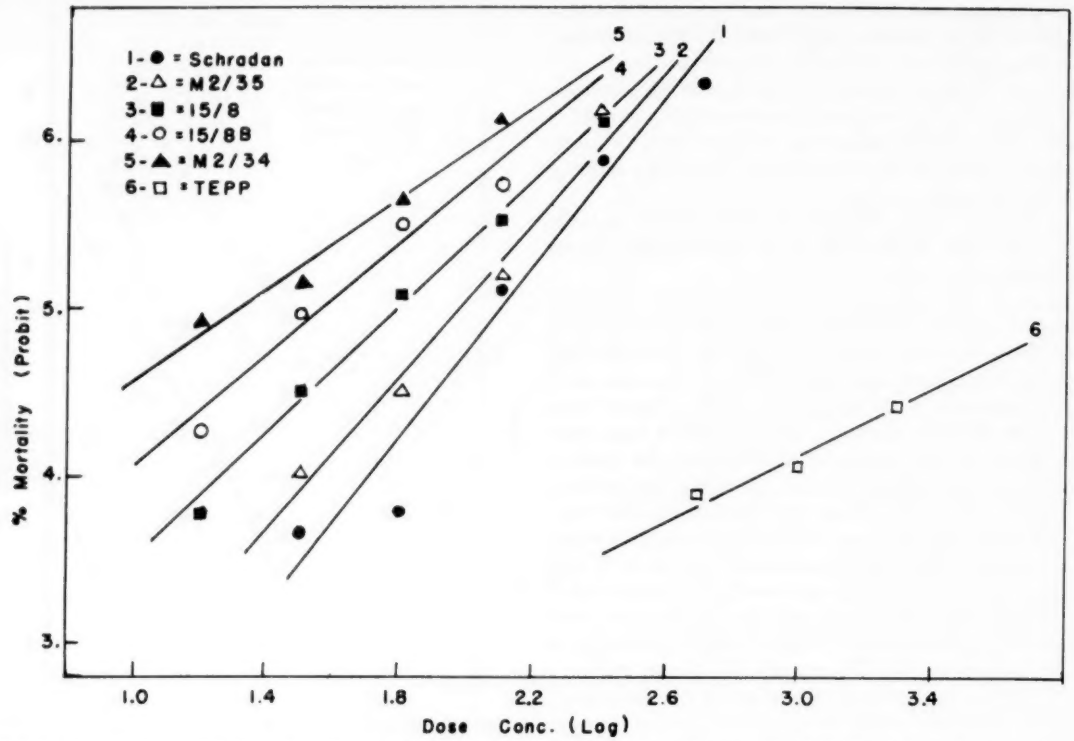


FIG. 9.—Comparison of Series IV materials (Pyrophosphates) against chrysanthemum aphid based on systemic action.

temic action than analogous compounds with P—O—C linkage.

2. An increase in the length of the chain beyond two carbon atoms on either side of the sulfur linkage reduced the effectiveness of the esters. The reduction of contact effectiveness was about equal for groups on either side of the sulfur linkage, but the effect on systemic action was greater for a group between the sulfur and the ester linkages than for a group beyond the sulfur linkage.

3. The isopropyl group caused less change in effectiveness than n-propyl or higher alkyl groups.

4. A methyl group beyond the sulfur linkage tended to be less effective than an ethyl group by contact action but more effective systemically.

The pyrophosphate series showed the following trends:

1. Stepwise replacement of ester groups by dimethylamido groups resulted in a decrease in both contact and systemic effectiveness as well as in the rate of hydrolysis. Unsubstituted TEPP fell far below its ideal position in systemic effectiveness because of its extremely rapid deterioration by hydrolysis.

2. The rate of hydrolysis of TEPP and derivatives containing one and two (asymmetrical) dimethylamido groups was rapid enough to limit their practical use as systemic insecticides.

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The Possible Use of a Polyhedrosis Virus in the Control of the Great Basin Tent Caterpillar[1]

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The results of preliminary tests of application by ground equipment of a polyhedrosis virus to control the Great Basin tent caterpillar, *Malacosoma fragile* (Stretch), were reported in 1954 (Clark & Thompson). In these tests, the virus was applied in concentrations that ranged from 0.5×10^6 polyhedra/ml. suspensions to 13×10^6 polyhedra/ml. suspensions, and at a rate of 10 gallons of suspension per acre for all plots (see table 1). All of the various concentrations applied resulted in virus infections with mortality rates high enough to be observable in 9 to 20 days.

As a result of the ground equipment tests, it was concluded that experimentation with commercial methods of application were warranted. The possibilities of controlling *M. fragile* with the virus as applied by aircraft were tested in 1954 and 1955. All the tests reported were conducted on bitterbrush, *Purshia tridentata* D.C. This low-growing, highly valued range browse species is economically the most important host of *M. fragile* in California.

1954 TESTS.—*Location.*—The 1954 tests were conducted in the vicinity of Truckee, Nevada County, California. The tent caterpillar has been noticeable in this area since about 1949, but has remained more or less static in scattered colonies without building to the heavy population densities which frequently characterize infestations of this insect. Field work with the virus has been conducted over various parts of this area since 1952. During this time close observation has shown that the incidence of naturally occurring virus infections has been very low; the disease has not been an important controlling factor, and, by outward appearance, has not been of sufficient intensity to influence the tests.

Plots.—As a result of the rather low density and scattered nature of the infestation, it was necessary to locate the plots where infestations occurred rather than select them in any random manner. Each of the four plots was 10 acres in size. Plot 1, a square, was laid out on the eastern edge of Boca Lake approximately $3\frac{1}{2}$ miles northeast of plot 4, the closest of the other three plots. Plots 3 and 4, each a rectangle, were located adjacent to each other in the form of a T at the southern end of the abandoned Truckee airport. Plot 2, a rectangle, lay about $\frac{1}{2}$ mile west of plots 3 and 4. Control or check sampling and observations were made in the area adjacent to each treated plot.

All of the plots were located in fairly dense bitterbrush stands that were free of an overstory of trees. Although the area in general supports a second-growth stand of ponderosa pine, the plots were selected to permit flying within 10 to 15 feet of the ground.

Concentrations and Rates of Application.—In the original design, it was intended to treat one plot at a rate of 2 gallons of 1×10^7 polyhedra/ml. suspension/acre; a second plot at 2 gallons of 1×10^6 polyhedra/ml. suspension/acre; a third plot at 5 gallons of 1×10^7 polyhedra/ml. suspension/acre; and the fourth at 5 gallons of 1×10^6 polyhedra/ml. suspension/acre. Inaccurate calibration of the spray equipment, however, prevented treatment according to plan and the plots received varying treatments which were determined by measuring the treated area after application. The actual treatments received by the plots are summarized in table 1. The four plots were treated on May 28, 1954, about 16 days after the onset of hatching.

Table 1.—Concentrations and rates of application of suspensions of polyhedra applied in field tests.

YEAR	PLOT	SIZE (ACRES)	GALLONS SUSPENSION	GALLONS ACRE	APPROXIMATE POLYHEDRA/ML
1953[a]	53-1	0.5	5	10	13×10^6
	53-2	.5	5	10	5×10^6
	53-3	.5	5	10	5×10^5
	53-4	.5	5	10	6×10^6
	53-5	.5	5	10	3×10^6
	53-6	.5	5	10	1.5×10^6
1954[b]	54-1	8	30	3.7	4.6×10^5
	54-2	8	60	6.2	6.9×10^5
	54-3	8	20	2.5	6.9×10^6
	54-4	7	50	7.1	8×10^6
1955[c]	55-1	50	100	2	5×10^5
	55-2	50	100	2	5×10^5

[a] Applied by ground equipment.
[b] Applied by airplane at 10 to 15 feet.
[c] Applied by airplane at 150 to 200 feet.

Sampling Methods.—In determining the results reported earlier, Clark & Thompson (1954) used comparative counts of larvae per tent or colony on treated and untreated plots. It was pointed out, however, that this method becomes ineffective with the scattering and diminished gregariousness of older larvae. The same method was used as long as possible in 1954. In addition to the counts of larvae per tent which were carried out on the 7th, 15th, 20th, and 32nd days following application, each tent taken in the sample was examined for virus-killed larvae. The presence or absence of one or more virus-killed caterpillars was recorded by tent following confirmation or rejection by light-microscope examination of field observations. As will be described in the following section, the induced virus-mortality on the plots was delayed beyond the period of accuracy of larval counts. This delay prompted attempts at two methods of determining comparative densities of pupae on the treated and untreated plots. Larvae of *M. fragile* pupate in several sites but most commonly at the ground surface and commonly in the abandoned tent.

To compare rates of pupation at the ground surface, 6×6-inch cardboard squares were distributed at 10- and

[1] Contribution from the Laboratory of Insect Pathology, Department of Biological Control, College of Agriculture, University of California. Accepted for publication February 28, 1956.

20-link intervals along a surveyor's chain laid through the plots. The squares were placed at these intervals regardless of the nature of the immediate ground cover, and were held in place by 16d nails pushed into the soil. Following pupation, the cards were collected and the number of pupae under each was recorded.

Comparative counts of pupae formed in the tents were made by opening and examining a number of tents selected at random through the treated or control area. Two hundred tents per plot or control area were examined unless no pupae were found in the first 200 taken. If none were found in the first 200, counts were continued until the first pupa was taken.

Results.—Polyhedrosis infections were induced by the aircraft application on all four plots. An interesting result of the 1954 trials was a delay in virus-caused mortality as compared with the 1953 applications of heavier concentrations and suspension. As was noted, this delay extended the period of mortality beyond the period of accurate larval counts and thus prevented conclusive comparisons between plots. The effects of the artificially distributed virus can be described in general with indications of specific plot differences noted.

The first evidence of polyhedrosis-caused mortality was observed on the 15th day following application. At this time, one or more virus-killed larvae or larval remnants were found in 5 of 10 tents on plots 1 and 3; 7 of 10 tents on plot 2; and 9 of 10 tents on plot 4. Only a few insects in each tent were dead, however, and the resulting changes in population were not great enough to be demonstrable by the sampling method used. In the total of 20 tents counted and examined in the control areas, none contained larvae or larval remnants dead of polyhedrosis.

The examination on the 20th day following application revealed a slight decrease in the proportion of tents on all plots showing virus-killed larvae. These figures were 3 of 10 tents on plots 2 and 3; 2 of 10 on plot 1; and 7 of 10 on plot 4. It is to be noted that the remains of young larvae dying on or in the tent frequently become lost in the tent debris or in new webbing. As a result, the records of tents with one or more dead larvae are frequently indicators of current and not cumulative mortality. They do not necessarily give any indication of mortality prior to the preceding 3 or 4 days. No reliable differences in comparative larval counts were obtained at this time.

On the 32nd day following application, widespread and high mortality was easily observable throughout all the plots. The proportion of tents with one or more current virus-killed larvae increased to 8 of 10 on plots 1 and 3; 9 of 10 on plot 2; and 10 of 10 on plot 4. Tents with all larvae dead of the disease, as shown in figure 1, were common on all treated areas. The average of comparative counts of larvae per tent on the 32nd day following application were 30, 30, 20, and 5 for plots 1, 2, 3, and 4, respectively, compared with 70 for the control areas (fig. 2). Due to the inaccuracies of the sampling procedure, however, these data are not good indicators of the real decline in population.

Following this examination no further larval counts were made. Careful observations indicated that high mortality continued through the remaining 3 to 4 weeks of larval development. Gross differences between treated and untreated areas were easily observable but could not be measured.

FIG. 1.—Dead larvae on a typical tent found on all 1954 plots 32 days following application. Virus-killed larvae are occasionally confused with normal exuviae, but can be distinguished by their weight and by microscopic examination.

On July 20, the cards for comparing density of pupae were laid out through the plots and control areas. At this time, most of the caterpillars were mature and wandering over the area as is characteristic in the pre-pupation period. In anticipation of heavier pupation on the untreated areas, and because this sampling technique was untested, 200 cards were placed on the check areas and 325 on the plots. On August 4 and 5, all the cards were picked up and examined.

Pupation under the cards was very light in all areas. A total of 12 pupae were found under the 200 cards on the check areas, distributed as 2, 3, 3, and 4 in the four areas. Only one was found under the 325 cards distributed over the four treated plots.

The counts of pupae formed within the tents were also made on the 4th and 5th of August. Summarized as one treatment, there was 1 pupa per 91.5 tents in the treated plots and 1 pupa per 9.2 tents in the control.

At various intervals through the period of observation, a total of 100 tents were selected at random from the check areas. The larvae in these tents were counted and each tent was carefully examined for larvae dead of polyhedrosis. None of the tents among these 100 contained

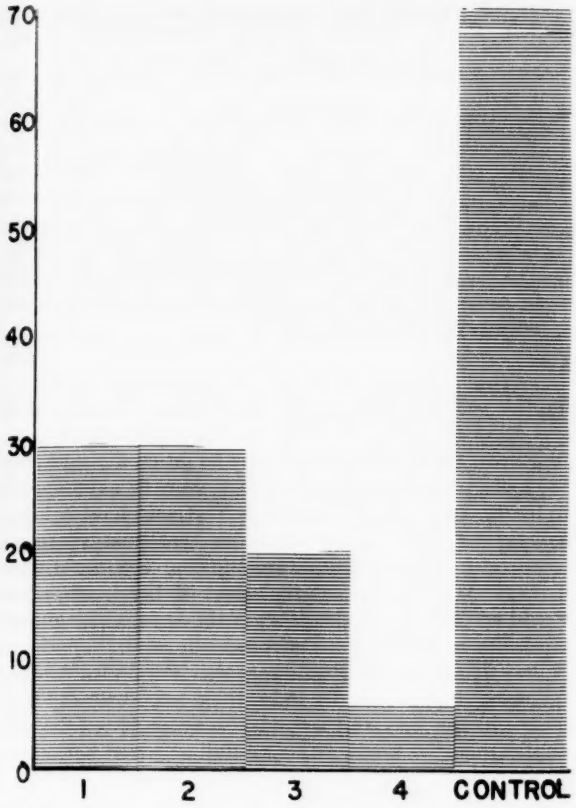

FIG. 2.—Comparative counts of larvae per tent, 1954 plots, 32 days following applications.

one or more virus-killed larvae. An extremely light scattering of virus-killed insects, below the point detectable by the sampling method, was observable through the area, however.

1955 TESTS.—Following the positive results of the 1954 aircraft-application tests, it was desirable to test applications of the virus under more severe conditions and to seek the lowest possible concentrations and rates of application effective in controlling *M. fragile*.

Bitterbrush, the economically important host of *M. fragile* in the eastside region of California, is frequently found in association with stands of ponderosa pine. Although large open stands of bitterbrush occur, the usual intermingling is such that aircraft application of any insecticidal material to bitterbrush is more typical of forest rather than rangeland spraying. This is true in regard to the height above the ground of the applying aircraft as well as to the amount of intercepting foliage. Plots were selected and treated to test applications of the virus under such conditions.

Location.—Two plots, each 50 acres in size, were located in the Badger Wells area of Modoc County, California. Both plots were located in *M. fragile* infestations of stands of bitterbrush growing under cutover ponderosa pine. The plots were located about 3 miles apart; control plots were about ½ mile from the respective treated plots. Both applications were flown above tree-tops at 150 to 200 feet from the ground.

Concentrations and Rates of Application.—A suspension of about 5×10^5 polyhedra/ml. was applied at the rate of 2 gallons per acre. No adjuvants were used. The applications were carried out at 6:00 A.M. on May 23 on which date most of the larvae were in the second instar.

Following application, the effective concentrations on both plots were reduced by a light rainfall during the night of May 23. A weather station within 6 miles of the plots recorded 0.06 inches precipitation.

Sampling Methods.—Following application, counts and observations similar to those described in the preceeding section were carried out through the period of larval development. The sampling procedure was modified in that prior to treatment 500 tents on one of the plots and 500 tents on the adjacent check area were marked with consecutively numbered tags. The 20 tents collected for the 5 weekly counts of larvae were then selected by drawing from a table of random numbers. The variation in light intensity on the tents under the stand of trees also seemed to have an effect on the sampling technique. The light intensity was uniform throughout the open plots of 1954, whereas light intensity on the bitterbrush under the pine cover on the 1955 plots varied with the shade cast by the trees. An apparent result of this intermittent shade was that the larvae of different tents seemed to be less uniform in congregating on or in the tent during the hot part of the afternoon when samples were taken than was found among the larvae of the fully exposed plots of 1954. Sullivan & Wellington (1953) have investigated the complex of reactions that may be involved in three species of *Malacosoma*.

RESULTS.—Little, if any, disease followed the 1955 applications. An examination of 100 tents per plot per week for 5 weeks revealed only one colony with one or more virus-killed larvae. In addition, a total of 30 colonies per week was collected from the two plots for counts of the larvae, and each of these colonies was examined for indications of polyhedrosis. None were found throughout the 5-week period of observation.

DISCUSSION.—*1954 Field Plots.*—From the data presented (figures 2, 3,) and from field observations, it can be concluded that aircraft application of concentrations and dosages indicated (table 1) brought about a reduction on all four of the 1954 treated plots. The degree of the virus-caused mortality at the conclusion of the tests appeared to be about the same as that obtained from ground application of heavier volumes in 1953 (Clark & Thompson 1954). The most interesting result of the 1954 tests is the increase in the period between application and mortality as compared to this period in the 1953 tests.

Population declines in the 1953 plots were observed by the 14th day after application on all six plots and appeared to reach a peak by or before the 22nd day. In contrast, noticeable declines in population as a result of the polyhedrosis treatment on the 1954 plots did not occur until after the 20th day following application. The peak of mortality was apparently not reached on any of the four 1954 plots until after the 32nd day. The causes of this delay are not known at this time, but certain variables such as the weather following application, an inverse dosage-incubation period relationship, and the possible transmission of the disease from larva-to-larva may be considered.

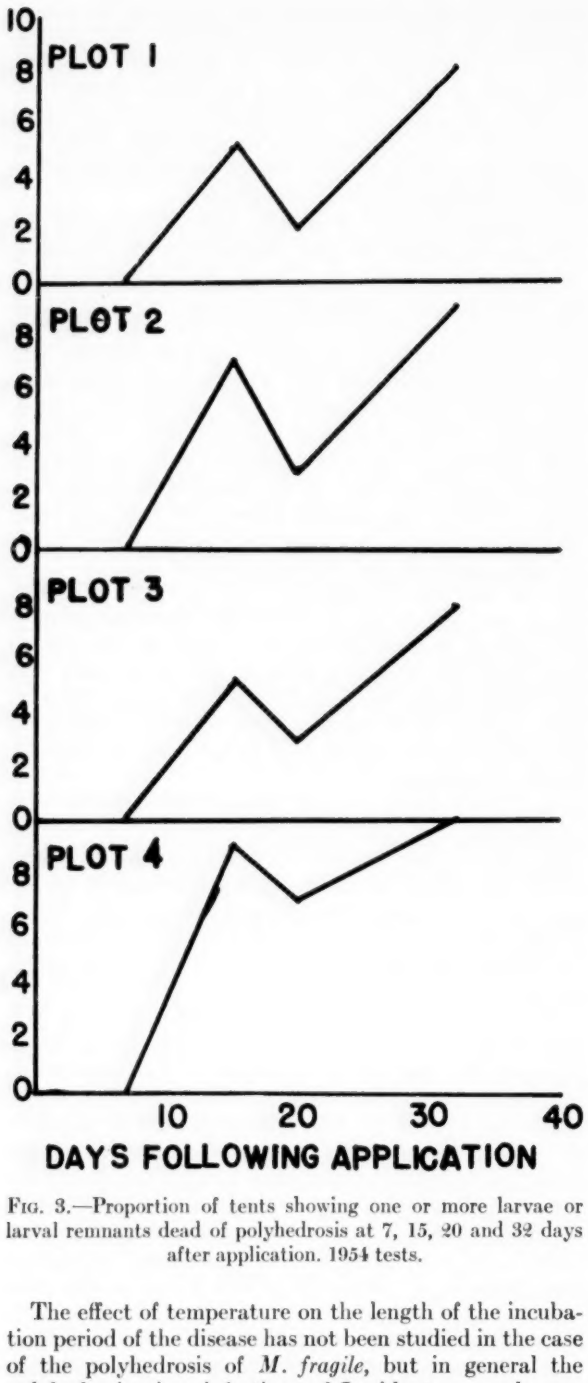

FIG. 3.—Proportion of tents showing one or more larvae or larval remnants dead of polyhedrosis at 7, 15, 20 and 32 days after application. 1954 tests.

The effect of temperature on the length of the incubation period of the disease has not been studied in the case of the polyhedrosis of *M. fragile*, but in general the polyhedrosis virus infections of Lepidoptera are known to proceed more slowly at lower temperatures (Steinhaus 1949, Thompson & Steinhaus 1950, Bergold 1953, Tanada 1953). In considering marked differences in the length of time between application and death in comparative field trials of a polyhedrosis of the European pine sawfly, *Neodiprion sertifer* (Geoffr.), Franz & Niklas (1954) suggested that the differences they observed were caused by unseasonable cold and rainfall following the earliest application.

Comparative data on temperature and precipitation

Table 2.—Temperature and precipitation data from Boca Station, U. S. Weather Bureau, Climatological Data,[a] California. U. S. Department of Commerce.

Days Following Application Date	Temperature (°F.)						Precipitation (In.)	
	1953		1954		1954 Compared with 1953		1953	1954
	Ave. Max.	Ave. Min.	Ave. Max.	Ave. Min.	Ave. Max.	Ave. Min.	1953	1954
1st to 10th	74.5	29.3	65.4	27.7	−10	−0.6	0	0.13
11th to 20th	85.9	37.9	62.2	31.6	−23.7	−6.3	0	.13
21st to 30th	86.0	37.9	81.7	33.4	− 4.3	−4.5	.06	.05

[a] Volumes 57 and 58, numbers 5, 6, and 7.

for the periods following the application dates in 1953 and 1954 are given in table 2. The colder 1954 post-treatment temperatures were due to an earlier spring in 1954 which prompted treatments on May 28 as compared to treatments on June 24 in 1953. The considerably lower averages of daily maxima indicate that post-treatment temperature did tend to retard the course of the disease in 1954. Whether or not the effect of the temperature would account for all the delay in the appearance of high mortality is not known. Differences in rainfall appear to be less significant. In 1953, there was no rain for 22 days following application. In 1954, there was 0.13 and 0.18 inches in the first and second 10-day periods following application, but none of this rainfall occurred before the seventh day.

Bergold (1953) stated that, in general, decreased dosages of insect viruses increase the period from ingestion to death of the larva. Tanada (1953) studying this question in the granulosis of *Pieris rapae* (L.) found a gradual increase in this period with decreasing dosages; and Bird (1954) has indicated the same effect in the polyhedrosis of *Diprion hercyniae* (Htg.).

Laboratory tests in which larvae of *M. fragile* were fed a heavy concentration of polyhedral bodies indicated a range in incubation period of 8 to 20 days. This period is both longer and more variable than that reported for many virus diseases of insects. In particular, the incubation period for the granulosis of *P. rapae* is about 2 to 7 days with a high inoculum concentration at room temperature (Tanada 1953) and from 5 to 10 (usually 7) days for the polyhedrosis of *Colias philodice eurytheme* Bdvil. (Steinhaus 1948). In view of the longer and more variable incubation period in the polyhedrosis of *M. fragile* it appears likely that an increase in incubation period from decreased dosages would be more marked than in species with a shorter period.

Direct comparisions of dosages between the 1953 and 1954 treatments are not possible because of the differences in application method. In general, however, the total number of polyhedra applied per unit area were lower in 1954, the total gallons of suspension were also lower in 1954, and the 1954 application by aircraft probably decreased the effective concentration still further. The data collected in both 1953 and 1954 on the numbers of larvae per tent do not permit critical comparisons between the plots of a given series; but they do suggest that for each plot series, mortality on the plot receiving the heaviest dosage was somewhat advanced over the other

plots within the series (fig. 2 and figs. 1 and 2, Clark & Thompson 1954).

It is also possible to speculate that the delay in mortality was due to transmission of the disease from a few larvae directly infected by the spray application. Such a speculation assumes that the 1954 applications resulted in lethal infections in only a small proportion of the caterpillar population, and that these directly infected larvae served as foci of the disease. Clark (1955) reported tests indicating that such larva-to-larva transmission can occur in the polyhedrosis of *M. fragile*; Bird (1954) found that such transmission of the virus of *N. sertifer* rapidly increased the incidence of the disease during a single season.

A certain amount of support is lent to this speculation by the data on the number of tents showing one or more larvae dead of polyhedrosis (fig. 3). As was pointed out in a preceding section, this information indicates current rather than cumulative mortality during the early stages of an epizootic. As indicated in fig. 3, these data suggest a drop in current mortality on all plots between the 15th and the 20th day following application and an increase between the 20th and the 32nd days. While these figures are based on a sample of only 10 tents per plot, the pattern is the same for all four plots. Collecting the data for all plots, the number of tents of 40 showing one or more diseased larvae is 26, 15, and 35 for the 15th, 20th, and 32nd days, respectively. The decrease in mortality following the 20th day may suggest that a large part of the caterpillar population was infected secondarily and that these larvae were in a period of disease-incubation around 20 days following application.

It is recognized that the three factors discussed as possibly contributing to the delay in mortality—low temperatures, inverse dosage-incubation period relationship, and larva-to-larva transmission—are not mutually exclusive. All would tend to retard the course of the disease, and all may have been effective to one extent or another.

1955 Field Plots.—The results of the 1955 tests indicate that the virus dosage deposited on the plant and available to the larvae was too low to initiate infection. As described, the sampling and observational methods were very intensive and were designed in the light of previous experience to detect very low rates of infection. Apparently no infection was induced. It appears, then, that the concentration of the virus will have to be substantially increased for applications under the described conditions of the 1955 tests.

PROBLEMS INVOLVED IN THE APPLICATION OF INSECT VIRUSES.—One of the principle objectives of the present study is the development of a method of reducing populations of *M. fragile* that is economical, has a selective or directed effect on the ecology of the insect and associated species, and involves no residue hazard for the wild and domestic grazing animals that are on the range during the period in which control action against the insect must be taken. A long-term objective is the study of possible carry-over of the virus resulting in prolonged suppression of the insect population (see Clark & Thompson 1954, Clark 1955). Bird (1954) has stated that this prolonged suppression can be achieved with the introduction of the polyhedrosis of *Neodiprion sertifer*.

Since the inception of the study in 1950, there have been no economic populations of *M. fragile* in California. None of the results of the various tests, then, can be described in terms of prevention of damage to host plants. It is possible, however, to review certain problems and technical difficulties which have been encountered during the study and with which we will be concerned in the future development of the method. In the following discussion, the problems encountered in the *M. fragile* study are incorporated into the more general question of the use of the polyhedrosis viruses against susceptible insect species. In the past few years, there have been several reports of experiments on the artificial distribution of viruses against various insect pests and the technical difficulties brought to light thus far are discussed here in relation to the Great Basin tent caterpillar investigation.

Virus Supply.—While much of our study is concerned with concentrations and rates of application of the virus, dosages are important to us, in the applied sense, only in relation to the ease or difficulty with which we can obtain a supply of the pathogen. Thompson & Steinhaus (1950) in their study of the polyhedrosis of the alfalfa caterpillar, *Colias philodice eurytheme* Bdvl., suggested two means of producing large quantities of the virus. One method consisted of rearing large numbers of larvae in the insectary, infecting them with polyhedrosis, and collecting the larvae at or just before the time of death. The second method involved the use of a net in sweeping the dying larvae from a previously treated infested alfalfa field at the appropriate time following application. The second method has proven to be more practical and in this case experience has shown that virus-supply is not a limiting factor in the application of the method. Dosages are relatively unimportant in the use of the *Colias* virus because quantities far in excess of the need are inexpensively obtained. Bird (1950) reported a successful method of propagating the virus of *N. sertifer*. His method consists simply of spraying several thousand larvae massed in a collection tray. Bird also stated that two men could recover about 6,000 diseased larvae per day under the trees in a treated area. In the case of the polyhedrosis of *N. sertifer*, it appears that the collection methods Bird describes are sufficient to meet the relatively low dosages needed to control this insect. On the other hand, Bird (1955) found that the scale of virus production necessitated by high dosages required may make impractical the use of the virus of the jack-pine sawfly, *Neopidrion pratti banksianae* Roh.

A completely successful technique of recovering large quantities of the virus of *M. fragile* has not been developed to date. Three difficulties have been encountered. The nature of the host plants and the characteristics of the insect preclude the use of insect sweeping nets and similar collecting devices. Collection of diseased larvae has involved hand-picking in either field or laboratory rearings. Secondly, the long and variable incubation period of the disease results in a relatively low proportion of a group of larvae—infected and reared under the same conditions—arriving at a given state of disease at a given time. Contrasted to *Colias* larvae, among which perhaps more than 90% will be in a suitable condition for virus collection at the appropriate time after treatment, relatively few *M. fragile* larvae will arrive at this

condition simultaneously—the rest being in a too advanced or too retarded state of disease. The third factor is the resistance to infection which field trials indicate develops rapidly with larval growth. As a consequence of this increase in resistance, it has been found that field infections for virus production are most satisfactorily carried out with young larvae. The treatment of young larvae, however, as a consequence of smaller size at the time of death, returns a much smaller volume of virus per individual caterpillar than can be obtained from larval species that are susceptible in advanced stages of development.

These factors in virus production have prompted the attempts to find the minimum dosages required and improvement in methods of production. Progress to date suggests that adequate material for the treatment of several hundred or perhaps a few thousand acres is obtainable; but the use of the virus on the extremely large areas which frequently characterize infestations of *M. fragile* may ultimately depend on our ability to produce the very large quantities of virus required.

Timing of Application.—Thompson & Steinhaus (1950) state that in the virus-control of the alfalfa caterpillar, proper timing of the application is critical in the success or failure of the method. The critical nature of proper timing arises from the fact that the number of days between the time at which the seriousness of the infestation can be assessed and the occurrence of economic damage is only slightly greater than the number of days between application of the pathogen and the cessation of feeding by the diseased insects. Delayed applications, then, may cause high mortality, but this mortality may be too late to prevent serious damage to the crop. In 1950, Thompson & Steinhaus felt that proper timing was so important that practical application of the method might require supervision of some entomological competence.

Bird (1954) reported that to produce the greatest mortality, the virus of *N. sertifer* should be applied about the time of hatching of the eggs, but that serious defoliation can be prevented by application before the larvae have developed to the fourth instar. Dowden & Girth (1953) also obtained equally good results when the virus of *N. sertifer* was applied when about 90% of the eggs had hatched as well as 14 days later when most of the larvae were about three-quarters grown.

Although field work to date suggests that best results may be obtained by application of the virus within 18 days following the onset of hatching of *M. fragile*, this requirement does not appear to offer any serious obstacle to the use of the method. Ordinarily economic infestations can be assessed during the year prior to treatment. Additionally, the development of the larvae of *M. fragile* in the Truckee area of California requires 60 to 70 days (Clark 1956). Since virtually all of the damage caused by the pest occurs during the final 10 to 15 days of this period, the relatively long incubation period of the disease does not become critical.

It is interesting to consider this point in relation to both the applied aspects of the study and to the ecological role of the polyhedrosis of the closely related species, *Malacosoma disstria* Hbn. and *M. americanum* (F.). The larval development period of both of these species is significantly shorter than the 60 to 70 days required for *M. fragile* in the Truckee area. According to Hodson (1941), this period is about 35 days for *M. disstria* in Minnesota, and Schaffner (1950) quoted about 6 weeks for *M. americanum*. If the incubation period of and resistance to the disease increase with larval age in *M. disstria* and *M. americanum* in proportion to these increases in *M. fragile*, it would appear that the highly susceptible period in larval development is passed much more rapidly than in *M. fragile*. This would suggest that the timing of application of the disease against these two species might be highly critical. These same factors would also appear to reduce the rate of larva-to-larva transmission in naturally occurring epizootics. This may suggest that the polyhedroses of *M. disstria* and *M. americanum* are of lesser ecological importance than the polyhedrosis of *M. fragile*.

Application Techniques.—In certain studies in applied insect pathology, such as that on the alfalfa caterpillar, economic and cultural conditions, and the availability of large volumes of the virus permit the use of high concentrations at high rates of application of the suspension. These circumstances do not require refinement of application techniques to secure the maximum effectiveness of the virus applied. In the study of the control of *M. fragile*, on the other hand, a highly desirable economic objective is the application of the minimum gallons of suspension per acre with the minimum concentration of polyhedral bodies necessary to achieve control. Arriving at this objective will require using application techniques which secure the maximum results from the material available. At the present time, little of the empirical information needed seems to be available. The influences of such factors as spray distribution of suspensions of the protein envelopes within which the virus rods are occluded, suspension adjuvants, and the optimal relationships between volumes and concentrations are largely unknown. The need for obtaining and using information bearing on such points will vary with the particular insect problem under study.

SUMMARY.—During 1954, tests of the aircraft application of the polyhedrosis virus of the Great Basin tent caterpillar, *Malacosoma fragile* (Stretch) were conducted near Truckee, California. These applications, made from a low-flying plane over infested bitterbrush, resulted in high mortality of the *M. fragile* population. This mortality was retarded, however, for several days beyond that experienced with application by ground equipment. The possible role of three factors—low temperatures, low dosages, and larva-to-larva transmission—in this delay in mortality are discussed.

In 1955, the virus was tested under more severe conditions. Very low concentrations were applied to two 50-acre plots in Modoc County from a plane flying 150 to 200 feet above the ground. These plots were located on infested bitterbrush growing under a cutover stand of ponderosa pine. Both plots received a light rainfall during the night following application. Apparently the effective dosage deposited on the host plant was too low to induce an epizootic. No disease could be detected by intensive sampling maintained for 5 weeks following application.

Technical problems that have been encountered in the study of the use of the virus in the control of *M. fragile*

are discussed in relation to the more general question of the use of the polyhedrosis viruses against various susceptible insect species. The principal problem now at hand in the study is the procuring of large quantities of the virus. The proper timing of the application does not appear to offer a serious obstacle to the use of the virus. The shorter periods of larval development of *M. disstria* and *M. americanum* suggest an interesting difference between these two species and *M. fragile* in respect to application timing and the ecological role of the polyhedroses. Empirical information on application techniques to secure the maximum effectiveness of the virus available is needed.

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A Simplified Quantitative Olfactometer for Use with Agriculturally Important Insects[1]

WILLIAM F. CHAMBERLAIN[2]

The study of repellent materials used against insects shows that a great deal of work has been conducted on the pests of man and animals, but very little, by comparison, on the pests attacking the plants man and his domestic animals use for food. In general, crop repellents have not proved as successful as crop insecticides or the repellents man uses on himself or his animals. This does not mean that a good repellent for agricultural crops will never be found. It only indicates that our present state of knowledge of agricultural repellents compares with our knowledge of insecticides before the advent of DDT and the other synthetic organic insecticides. More studies of repellents, both in the field and in the laboratory with olfactometers, will eventually develop a satisfactory agricultural repellent. Preliminary work with a simplified quantitative olfactometer with crop pests is reported in this paper.

A great many repellent candidate materials have been tested against crop pests by other investigators. Investigations of possible repellents have often started by testing extracts of plants known to be immune to the attack of one or more species of insects. One of the most extensive studies of this type is that of Metzger & Grant (1932). In an attempt to find a repellent for the Japanese beetle, extracts were made of 390 species of plants not attacked by the Japanese beetle. Many of the materials that have shown repellent qualities probably do so because of the physical nature of the material. Materials such as Bordeaux mixture leave a whitish deposit, which undoubtedly accounts in part for the repellency ascribed to this material. Dethier (1947) lists many of the materials that have shown some repellent qualities.

EQUIPMENT.—The standard procedure in testing materials for repellency has been to apply the test material

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to a plant and later determine the degree to which the plant escaped attack by an insect pest. This process must be the ultimate test of any repellent material, but many possible repellents have been rejected because of the severe burning of the foliage. Chamberlain (1953) was able to circumvent the usual burning caused by pine products when sprayed on corn, by incorporating the repellent into a dust.

Another means of testing for possible crop repellents is to conduct experiments with an olfactometer. The earliest use of an olfactometer to study insect reaction to odor was by Barrows (1907). He introduced the simple Y-tube. In this type of apparatus the insect was introduced at the base of the Y, and at the fork could make a choice between the two arms. One of the most thorough pieces of research conducted with this type of olfactometer is that of McIndoo (1926) on the reactions of potato beetles. A disadvantage of the Y-tube olfactometer is that some source of strong attraction is needed in order to obtain the active participation of the insects in a test. Often a strong light served as the attractive force.

Orginally when experiments were started in this laboratory, this type of apparatus was tried, but because the active participation of only a few species of insects could be obtained, its use was discontinued. Another type of olfactometer is that developed by Folsom (1931) which was called a chemotropometer. This instrument consisted of a simple straight tube and it served very well for the purpose of studying attractants, but its use with repellents is of doubtful value as a tube of this sort soon becomes completely filled with the odor of the repellent. With both the Y-tube and the straight tube, the control of the concentration of a repellent is either difficult or impossible.

A type of instrument that requires the active participation of nearly all the individuals and can be used with a variety of insects is that used by Wieting & Hoskins (1939) or the modification used by Willis & Roth (1950). Also, the concentration of the chemical can be controlled by the use of a series of flowmeters. An instrument of this type (figure 1) has been used in the tests reported below, but considerable modification has been made. The modifications insured that the test insects were all subjected to an odor gradient, and at the same time some degree of simplification has been attempted so that the cost of building the olfactometer was reduced. A source of air was supplied from a pressure pump. The air, by flowing through the bottle at point A, could be humidified as desired, depending on the design of the experiment. If the air was to be humidified, the bottle was partly filled with water. The air then entered the distributing system at point B and the total flow of air through the rest of the instrument was controlled by use of a pinch cock (C) on a rubber tube attached to the distributor. The air was then distributed through three flowmeters (E) each with a separate stopcock (D) so that the flow through each flowmeter was regulated. Each flowmeter was marked with a centimeter scale from 0 to 10. In operation, a differential pressure of 20 cm. was possible. The three flowmeters were filled to the zero line with a dyed oil of 0.851 specific gravity. Each of the three flowmeters had a capacity of 471 liters of air per hour at 80 degrees F. at the 10-cm. setting.

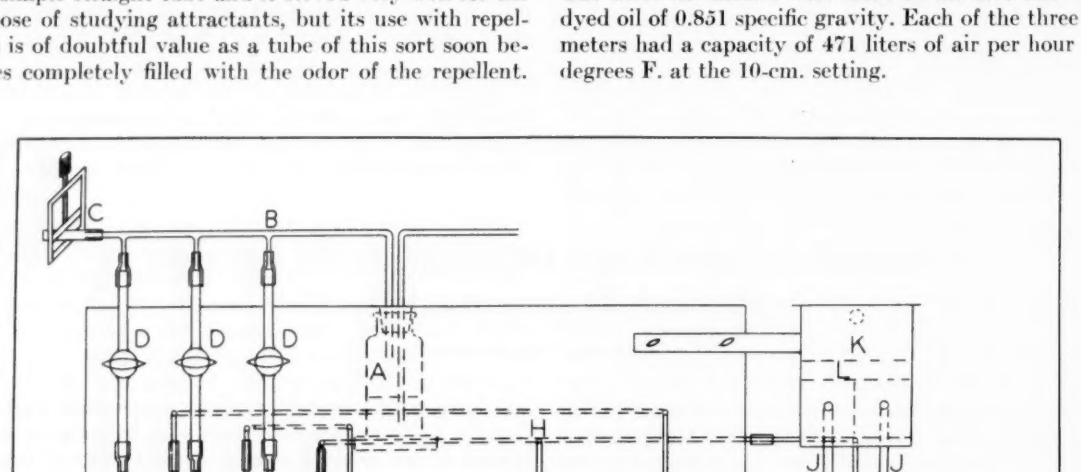

FIG. 1.—Diagram of the olfactometer used in the tests with agricultural insects. Key: (A) humidifying bottle; (B) glass tube distributor; (C) screw clamp; (D) glass stopcocks; (E) flowmeters; (F) bottle containing odor; (G) T stopcock; (H and I) glass Ts; (J) inlet tubes to olfactometer; (K) olfactometer; (L) test cage.

Air from the center flowmeter passed by means of glass tubing through the bottle (F) or U-tube containing the source of odor. All the experiments reported in this paper were conducted using the bottle as the odorizer, but ocasionally in recent experiments a U-tube partly filled with both the glass beads and the chemical to be tested has been used. The U-tube materially reduces the amount of chemical needed to conduct a test, and it can also be used in experiments to determine the effect of the continuous flow of air over surfaces treated with a repellent, thus simulating field conditions. When either the bottle or U-tube was used, the loss of chemical with time was determined by weighing before and after a test run.

Another easily made modification is the comparison of one test chemical against another. By inserting a fourth flowmeter and a second t-cock, the comparison between the two chemicals at varying concentrations can be made.

Odorized air passed from the odorizer bottle to a t-type stopcock (G) where it was diverted either to the right or left side of the olfactometer. Air flowing from either the left or right flowmeter was mixed with the odorized air at H or I. In this manner nearly any desired dilution of the odor was obtained.

The olfactometer (K) consisted of a stainless steel box measuring $7\frac{1}{2}$ inches high, 6 inches wide and 3 inches deep. The top was covered with a piece of window glass or sheet plastic, and during operation with a black cloth. The bottom had two $1\frac{1}{2}$ inch round openings covered with window glass sealed in place. The bottom half was divided by a stainless steel partition and the two holes in the bottom were centered in each of the smaller compartments thus formed by the partition. Insects to be tested were confined in a small cage at the position L. An enlargement of the cage is shown as the insert L. This small cage measured $5\frac{3}{4}\times2\frac{3}{4}\times1$ inches. The sides were of stainless steel, while the top and bottom were covered with 20-mesh brass screen. If extremely small insects were tested, the top and bottom of the cage were covered with even finer mesh screen. On one side of the cage was an opening for inserting or removing the insects. In operation, the cage was placed in the olfactometer so that one screened surface rested directly on the top of the partition dividing the lower part of the olfactometer. Small tabs at the same level as the top of this partition helped support the cage. With the use of two square ports, all of the insects in the cage must of necessity be exposed to air from either the right or left inlet to the olfactometer. The inlets were located on the back of the instrument and each was centered $1\frac{1}{2}$ inches from the side and bottom. The stainless steel tubes (J) which carried the air into the instrument continued inside to the center of each of the lower compartments and then turned downward for $\frac{3}{4}$ inch before ending. Air thus entering the olfactometer was evenly distributed over the complete cross section of each compartment. Tests with ammonium chloride smoke showed that the air rose in two square columns, and at the top of the partition dividing the lower part of the instrument the air continued in two columns through the cage as long as the air flow was 5 liters or more per minute through each inlet port. A sharp straight boundary line was formed between the two quantities of air treated in different ways. Below 5 liters per minute, the boundary inside the test cages was less sharp. Air in the instrument was exhausted to the outside of the test room through a single opening in the top of the olfactometer. There were two symetrically placed 60-watt incandescent lights below the olfactometer which silhouetted the insects during observations which were made from the top.

The complete instrument was kept in an air conditioned room where the temperature was maintained between 80 and 85 degrees F. In order to maintain the same flow of air through both the right and left sides of the olfactometer, it was first necessary to determine the flow of air through the flowmeter at different pressure differentials. It was necessary to make determinations at different pressure differentials since equivalent increment changes of pressure did not give equal changes in the flow of air. Two different methods were used to determine the air flow. The first method employed the Pitot tube which, in principle, consists of a small diameter tube which is placed so that the tip is in the same plane as the exhaust port of the flowmeter and centrally located. The tip of the Pitot tube should be less than one-tenth the size of the exhaust port. The small diameter tube is then connected to a U-tube partly filled with a liquid. In these experiments, the oil used in the flowmeters was used in the U-tube. The exhaust air from the flowmeter caused a differential pressure in the U-tube. Knowing the diameter of the exhaust port of the flowmeter, the differential pressure in the U-tube and the specific gravity of the oil, the volume of air per minute was calculated for any setting of the flowmeter. Two formulae were required. The first $V=\sqrt{2gh}$ determined the flow of air in feet per minute. In this formula g is the acceleration of gravity, 32.1 feet per second, and h is the weight of a column of air equivalent to the oil displaced in the U-tube. Since, in these experiments, we were interested in liters of air per minute, the above formula was converted to mm. per second by multiplying the $\sqrt{2gh}$ by 12(25.4). In order to convert mm. per second to liters per minute, the second formula was used. In the formula $Q=VA$, Q is the volume, V is the velocity, and A is the cross section area of the exhaust port of the flowmeter.

Using the Pitot tube the flow of air was determined for 4, 6, 8, and 10 cms. In figure 2 these values are plotted in order to obtain the complete curve.

In the second method used to determine the flow of air a 1-liter graduated cylinder was inverted in a tank of water. Air was pumped from the flowmeter to the cylinder and the time required to pump 1 liter was recorded. During the time the air was being pumped into the cylinder, the water level inside and outside the cylinder was kept at the same level.

Figure 2 shows the results of determination of air flow by the two methods. With either method there are inherent errors. The oil displacement in the U part of the Pitot tube never measured more than 1 centimeter so that accurate reading to a fraction of a mm. was necessary in order to obtain accurate results. Also, even though the area of the Pitot tube tip was small, a slight effect was expected. In the water displacement method, frictional loss and the need to maintain the water level at exactly the same level inside and outside the cylinder may cause a slight error.

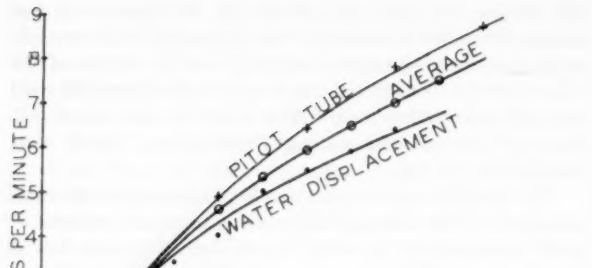

FIG. 2.—Variations in the air flow when the air pressure is changed. The pressure is expressed as the difference in the levels of the oil in the manometer.

Since the need for extreme accuracy was not necessary in the routine testing of repellents, a value for the flow of air between the Pitot tube and the water displacement method was used to obtain equivalent settings of either the right or left side of the olfactometer. This average value was also used to determine concentration of repellent in the air.

METHODS.—Insects tested in the olfactometer were collected in the field as needed and were always used within a short period after collection. If the test insects were extremely active or could be damaged by struggling, they were first anesthesized with CO_2 gas. In the tests reported in this paper, 14 were placed in small test cages but in recent tests, 20 are being used. The cages were held in the test room until the insects revived. At this time, 50 ml. of the test chemical was placed in the bottle used to odorize the air. The bottle with the test chemical was then weighed and replaced in the line from the center flowmeter. On revival of the insects in a test cage, they were placed in the olfactometer, the glass cover and cloth put in place, and the streams of air started through the olfactometer. The settings on the flowmeters were determined by the graph (Figure 2), so that both ports of the olfactometer received the same flow of air.

After 5 minutes the black cloth was removed, the lights below the olfactometer were turned on, and a reading was made on the distribution of the insects. The black cloth was then replaced and the lights turned off. At the end of 10 minutes, a second reading was taken. At this time the flow of odorized air was changed to the left side of the flowmeter by means of the T-cock, and the necessary adjustments of the flowmeters were made. At the end of another 5 and 10 minutes, the third and fourth readings were made. Results of any test in which a toxic or paralyzing effect was noted were discarded. The only material that occasionally had a paralyzing effect was the amyl acetate at higher concentrations.

Willis & Roth (1950) showed that beetles have nearly completely reorientated within 2 minutes after reversal of flow of air. Since a 5-minute reading period was used in these tests, no adjustment period was necessary. Contamination at reversal cannot be considered of importance in these tests as all the materials were relatively volatile. In later tests, not reported in this paper, a 2-minute reading period was used along with an adjustment period at reversal of air flow.

After completion of the 20-minute test, the bottle with the test chemical was again weighed. The test cage was removed and a second test cage with insects was inserted in the olfactometer. The same chemical may be tested again or another chemical may be tested. Using this instrument the following insects have been tested: fourth and fifth instar nymphs of the red-legged grasshopper, *Melanoplus femur-rubrum* (DeG.); adults of the corn earworm, *Heliothis zea* (Boddie); adults of the American cockroach, *Periplaneta americana* (L.); adults of the German cockroach, *Blattella germanica* (L.); adults of a common green pentatomid, *Thyanta custator* (Fab.); adults of a clover weevil, *Hypera meles* (Fab.); and adults of the four-spotted tree cricket, *Oecanthus nigricornis quadripunctatus* Beut.

Only the results with the red-legged grasshopper, the corn earworm, and *Thyanta custator* are recorded in this paper as the results with the other species are incomplete. Only a relatively small number of chemicals have been tested to date, but they have been sufficient to show that the instrument is capable of testing a variety of chemicals. Nearly all are products or fractions taken from turpentine. In addition to the turpentine products or fractions, dimethyl phthalate, amyl acetate, and diethyl toluamide have been tested.

RESULTS.—In order to compare different chemicals as to repellency, some standard method of calculation must be used. The method used by Willis & Roth (1950) of calculating an index number is very satisfactory. The index number of a chemical is determined by the formula: $I=(R-A)100/N$ where I is the index number, R is the total number of insects on the side of the olfactometer having no odor, A is the total number of insects on the side of the olfactometer being odorized, and N is the total number of insects times the number of recordings taken. Since most of the materials studied acted as repellents, repellency was taken as the positive value while attraction was the negative value. Using this formula, a chemical could have a possible value of -100 to $+100$. The closer a value is to -100 the greater its attractive value, while the closer it is to $+100$ the greater its repellent value.

The results with the chemicals tested as repellents are shown in tables 1 and 2. In all the tests recorded in these tables, the air flow was 8 liters per minute through each port of the olfactometer. In table 1, the air coming from the bottle with the test chemical was mixed with three times as much unodorized air before entering the olfactometer. In this manner the total concentration of chemical in the air was reduced. In table 2 the air passing through the bottle with the test chemical made up the total air supplied to one port of the olfactometer. It will be noted that although the total air passing through the test bottle was reduced to one-fourth in the results shown in table 1, the actual average quantity of chemical was only reduced about one-half below that of the results in table 2. This indicates that complete saturation of the air was not occurring when 8 liters of air passed through the test bottle per minute.

Table 1.—Comparison of repellents tested against *Melanoplus femur rubrum* with air from test bottle mixed with three times as much unodorized air.

MATERIAL	NUMBER OF TESTS	AVERAGE AMOUNT OF CHEMICAL IN MG./LITER/MIN.	AVERAGE INDEX NUMBER
Alpha pinene[a]	3	8.93	42.1
Camphene	3	6.32	41.3
Amyl acetate	2	13.6	40.0
Pine oil[b]	3	0.428	28.3
Solvenol 1[c]	3	2.90	22.8
Terpinyl acetate	3	0.422	11.3
Solvenol 2[d]	3	3.90	9.7
Dipentene 122[e]	4	3.24	9.5
Alpha terpineol[f]	3	0.250	5.6

[a] Hercules Powder Co., 85% alpha-pinene, 12% camphene.
[b] Hercules Powder Co., Yarmor 302 Pine oil, 65%-70% alpha terpineol. 10% dihydro-alpha-terpineol, 10% Borneol and fenchyl alcohols, 5%–10% other compounds.
[c] Hercules Powder Co., 30%–40% dipentene, 20%–25% para-menthane and paramethene, 10%–15% para-cymene, 10%–20% terpinolene, 5%–10% pinene, and 5%–10% other compounds.
[d] Hercules Powder Co., similar to solvenol-1, except slightly different proportions of constituents.
[e] Hercules Powder Co., 51% dipentene, 17% para-cymene, 14% para-menthene, 18% other compounds.
[f] Hercules Powder Co., 95% alpha-terpineol or related alcohols.

In general, compounds which were more volatile caused the greatest degree of repellency, and a reduction of the amount of chemical in the air reduced the index of repellency, but did not materially change the order of the chemicals in the tables. The study showed that when the concentration of dipentene 122 and solvenol 2 were reduced their repellency values dropped faster than alpha pinene or solvenol 1. This fact would seem to indicate that they would not be very effective repellents.

When a comparison was made between different insects, the red-legged grasshopper was slightly more sensitive than the corn earworm, and both of these insects were considerably more sensitive than *Thyanata custator*. It is interesting to note that the one well known repellent for mosquitoes, dimethyl phthalate, was a very poor repellent in the tests with the red-legged grasshopper and *Thyanata custator*. The three negative values in table 2, if they were of a greater order, would actually indicate attraction, but since they are so close to zero a better interpretation would be that they have neither an attractive nor repellent effect.

SUMMARY.—New modifications of currently used olfactometer apparatus are described which make this type of equipment more desirable for use in testing repellents against agriculturally important insects. The modifications reduce the cost of such apparatus and cause all the insects in an experiment to participate in the test. The essential part of the equipment consists of a rectangular stainless steel box with a smaller inserted cage containing the test insects. In the smaller test cage, the insects have a choice of the right or left port. This equipment can be used for testing a wide variety of insects. Quantitative control of the concentration of the chemical can be obtained.

Results of tests with a number of turpentine products or fractions along with several other chemicals were recorded. Tests have been conducted on eight species of insects in order to show the adaptability of the equipment.

Table 2.—Comparison of repellents tested with different species of insects with all the air of one port of the olfactometer passing through the test bottle.

MATERIAL	NUMBER OF TESTS	AVERAGE AMOUNT OF CHEMICAL IN MG./LITER/MIN.	AVERAGE INDEX NUMBER
Melanoplus femur-rubrum nymphs			
Alpha pinene	3	13.16	73.6
Amyl acetate	2	21.2	67.3
Solvenol 1	5	5.91	51.9
Dipentene 122	6	5.83	51.1
Solvenol 2	6	6.77	47.2
Terpinyl acetate	2	0.562	36.3
Terpinol 318	5	0.74	35.1
Pine oil	5	1.325	26.0
Alpha terpineol	3	0.958	22.3
Terposol 8	5	0.5	16.3
Dimethyl phthalate	4	0.69	3.5
Diethyl toluamide	4	1.499	1.9
Thyanta custator adults			
Dipentene 122	4	4.53	38.3
Alpha pinene	3	10.21	35.4
Pine oil	3	1.208	33.1
Solvenol 1	5	6.64	25.5
Solvenol 2	5	6.11	22.9
Terposol 8	2	0.562	20.0
Terpineol 318	5	0.583	11.5
Alpha terpineol	3	1.208	4.5
Diethyl toluamide	2	0.50	−2.1
Dimethyl phthalate	2	1.50	−7.1
Heliothis zea adults			
Alpha pinene	3	13.11	63.3
Solvenol 2	3	3.54	52.6
Dipentene 122	3	8.94	40.0
Solvenol 1	3	6.25	34.8
Alpha terpineol	1	0.50	11.1
Terposol 8	2	1.188	−5.5

Results with three species of insects tested showed that, in general, the more volatile compounds exhibited the greater degree of repellency. Alpha pinene, amyl acetate, the two "solvenol" fractions, and dipentene were among the most repellent materials. Some difference in repellency was exhibited by different species of insects.

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Effect of Lindane Emulsion Sprays on the Insect Invasion of White Pine Sawlogs and the Grade Yield of the Resulting Lumber[1]

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In experiments reported earlier on the application of BHC sprays to keep boring insects out of unseasoned pine logs, cordwood size material had been used and results were based entirely on the extent of insect damage to the logs (Becker 1955). In continuing the tests at the University's Mt. Toby Forest Reservation at Sunderland, Mass., pine sawlogs were used. In addition to being examined for insect damage, these logs were sawed into lumber, which was then graded according to standard grading rules of the Northeastern Lumber Manufacturer's Association.

Experimental Procedure.—Forty-two sawlogs were cut from living eastern white pines (*Pinus strobus* L.) in the early spring (March 23 to 27, 1953). Thirty were sprayed *only once* (April 8, 1953) before any insects attacked them, and then all were exposed to attack by borers. They were stored outdoors until autumn in a small sunny field surrounded by pine plantations. After periodic examinations of the bark surfaces throughout the summer, the bark was removed between October 9 and 15, 1953, and the various types of insect borings evident at the surface of the wood were tallied. Since all logs in the test were thoroughly examined, the data represent a 100% sampling of the logs. On October 20, 1953, the debarked logs were sawed into lumber (1610 board feet), and a few days later all the lumber was carefully graded.

Spray Treatments.—The small, powered, wheelbarrow sprayer had a pump capacity of 1½ gallons a minute. The nozzle contained a Number 3 disc and could be adjusted to produce a broad or narrow cone-shaped spray pattern. The spray output allowed ample time to obtain thorough coverage of a log without excessive waste of spray.

A 20% lindane emulsifiable liquid concentrate (commercially prepared) was diluted *in water* to three different strengths (0.4, 0.2, and 0.1% gamma isomer content by volume) and applied to three different groups of logs. Since lindane is the refined gamma isomer (the form having insecticidal action) of BHC from which the other isomers have been almost completely removed, in actual practice BHC emulsion sprays of equivalent gamma isomer content would be expected to be just as effective and much less expensive.

Each log was treated separately by carefully spraying the entire surface until the liquid began to run off. This required rolling each log along a skidway. An average of approximately 4¾ fluid ounces (141 ml.) of spray was applied per square foot of bark surface. The logs were dry before spraying, and no rain fell until after the spray had dried.

The lumbermen had left the logs lying side by side in a single layer on skids. The logs were treated in the order in which they lay because of the difficulty of handling logs of the size used. The 0.4% gamma isomer spray emulsion was applied to the first 10 logs at one end of the row, the 0.2% gamma spray to the next 10 logs, the 0.1% gamma spray to the next 10, and 12 unsprayed check logs were left at the other end. This order of application naturally resulted in some variations in the diameter, length, and board-foot content of the individual logs within each group and also between the four different groups of logs.

Methods of Sawing Logs.—In order to compare the actual effects of the different spray treatments, the sawyer was told to saw all logs in the same manner to produce the most lumber, regardless of whether they were damaged by insects or not. The logs were sawed into square-edge boards, 1-inch thick; and in the edging process, no attempt was made to cull out the sections of the boards that contained worm holes.

Insects Present.—Insect damage to the logs was divided into five broad groups; namely, round-headed borers, flat-headed borers, ambrosia beetles, bark weevils, and bark beetles. It was not always practicable to separate damage further. However, genera and species collected in the logs included[4]:

1. Round-headed borers (Cerambycidae): *Monochamus* sp., *Leptostylus* sp., *Acanthocinus obsoletus* (Oliv.), *Rhagium lineatum* Oliv.
2. Flat-headed borers (Buprestidae): *Chrysobothris* sp.
3. Ambrosia beetles (Scolytidae): *Gnathotrichus materiarius* Fitch.
4. Bark weevils (Curculionidae): *Pissodes approximatus* Hopk.
5. Bark beetles (Scolytidae): *Orthotomicus caelatus* (Eichh.), *Ips calligraphus* Germ., *Ips pini* (Say), *Polygraphus rufipennis* Ky., *Crypturgus* sp.

Results.—*Examination of Logs.*—A study of the insect infestation per square foot of bark within each of the four different groups of logs according to diameter class revealed no evidence to indicate a definite trend toward a heavier infestation of any of the five broad groups of insects in logs of either the smaller or larger diameter classes (7 to 15 inches) used in this test.

With regard to the total insect infestation in each of the four groups of logs, the 0.4% gamma isomer spray emulsion gave 98.7 to 100% prevention, whereas the 0.2% gamma spray gave 87.2 to 100% prevention against all types of insect damage. The 0.1% gamma spray gave 85.1 to 99.3% prevention against all insects except ambrosia beetles, against which it apparently failed in this test (table 1).

The percentages of control given for round-headed borers, flat-headed borers, and ambrosia beetles are based on the number of galleries *per square foot* that entered the wood; for bark weevils, it is the number of pupal cells *per square foot* at or adjacent to the cambium. Control for

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[4] Most insects were identified by specialists in the Section of Insect Detection and Identification, Entomological Research Branch, U. S. Depart. of Agriculture at Washington, D. C., a few were examined by Mr. C. A. Frost, Coleopterologist at Framingham, Massachusetts, and some by the senior author.

Table 1.—Prevention of borer infestation by spraying white pine sawlogs[a] with a lindane emulsifiable liquid diluted in water.

Per Cent Gamma Isomer Content by Volume	Bark Area (Sq. Ft.) and Log Content[b] (Bd. Ft.)	Galleries in Wood: Round-headed Borers		Galleries in Wood: Flat-headed Borers		Galleries in Wood: Abrosia Beetles		Galleries in Bark: Weevils (Pupal Cells at Cambium)		Galleries in Bark: Bark Beetles (Per Cent)	
		Number	Per Cent Control[c]	No.	Per Cent Control[c]	No.	Per Cent Control[c]	No.	Per Cent Control[c]	Area Infested	Control[d]
0.4	292.1 Sq. Ft. 474 Bd. Ft.	Mon.[e] 6 Others 0	98.7 100	0	100	1	99.9	2	99.8	Trace (on 2 logs)	99+
.2	269.6 Sq. Ft. 438 Bd. Ft.	Mon. 17 Others 1	96.0 99.9	0	100	118	87.2	13	98.9	Trace (on 7 logs)	99+
.1	244.5 Sq. Ft. 384 Bd. Ft.	Mon. 57 Others 6	85.1 99.3	41	94.8	870	0	64	93.9	3.6	93.1
Unsprayed	268.6 Sq. Ft. 314 Bd. Ft.	Mon. 420 Others 919	— —	873	—	919	—	1157	—	52.7	—

[a] Ten logs per treatment, 12 in unsprayed check.
[b] Board feet (Mill scale).
[c] Based on number per square foot of bark surface.
[d] Based on area infested per treatment.
[e] *Monochamus* sp.

bark beetles is based on the percentage of bark area covered by galleries *per treatment*.

Examination of Lumber.—The green lumber was carefully graded by qualified persons. Separate records were kept of downgrading due to worm holes as distinct from stain, decay, knots, wane (bark edges), and other defects that effect the grade of lumber. Each insect-damaged board was graded in two ways: first, as it would have been without the worm holes; second, the actual grade with the worm holes. Most eastern white pine lumber is graded as follows: D Select and better, Numbers 1 and 2 common (usually sold together), Number 3 common, Number 4 common, and Number 5 common. The better or select grades are understandably the most valuable, whereas Number 5 common, the lowest grade, is usually sold without profit.

In the lumber sawed from the unsprayed check logs (7 to 12 inches in diameter), no evidence was obtained to indicate that the logs of smaller diameter produced any larger percentage of boards downgraded because of insect damage than the logs of larger diameter used in this test. Logs of small diameter, of course, may contain a greater proportion of sapwood than the logs of larger diameter, and therefore may be susceptible to more damage by certain wood borers. Since the diameter variations in the three sprayed groups of logs were close to those in the unsprayed checks (7 to 15, 8 to 13 and 8 to 15 inches, respectively, in the logs sprayed with 0.4, 0.2, and 0.1% gamma isomer), it is felt that the variations in the sizes of the logs in the four groups had relatively little or no effect, in this test, on the extent of insect damage to the lumber.

The downgrading of lumber one or more grades to Number 5 common because of worm holes was 6.1% (4.0% down 1 grade and 2.1% down 2 grades) in the 0.4% gamma isomer treated logs (table 2). In the 0.2% gamma treatment it was 5.3% (all down 1 grade) and in the 0.1% gamma treatment it was 8.9% (5.5% down 1 grade and 3.4% down 2 grades). In comparison, downgrading in the unsprayed checks was 81.4% (58.7% down 1 grade, 17.9% down 2 grades, and 4.8% down 3 grades).

This downgrading (table 2) was attributed by the graders chiefly to "large worm holes" caused by round- and possibly some flat-headed borers rather than to the tiny "pin worm holes" caused by ambrosia beetles, even when the latter were present in the same board. According to the grading rules for white pine lumber, a very few large worm holes may be sufficient, in themselves, to warrant downgrading a board one or more grades to Number 5 common, whereas a few pin worm holes may sometimes be permitted in grades 3 and 4 common. These grading practices may help to explain why downgrading, to Number 5 common, of lumber from logs sprayed with 0.1% gamma isomer (in which there was

Table 2.—Volumes (board feet) and percentages of different grades of lumber sawed from white pine logs sprayed with a lindane emulsifiable liquid diluted in water.

Per Cent Gamma Isomer Content by Volume	Total Board Feet of Lumber	D Select and Better	No. 1 and No. 2 Common	No. 3 Common	No. 4 Common: Without Stain and Decay	No. 4 Common: With Stain and Decay	Number 5 Common: Due to Wane and Decay	Number 5 Common: Due to Worm Holes: No. Grades Downgraded 1	2	3	Number 5 Common: Due to Worm Holes: Total Downgraded	Total No. 5 Common
0.4	474	3[a] 0.6[b]	39 8.2	204 43.1	190 40.1	9 1.9	0 0	19 4.0	10 2.1	0 0	29 *6.1*	29 6.1
.2	438	6[a] 1.4[b]	87 20.0	180 41.0	132 30.0	0 0	10 2.3	23 5.3	0 0	0 0	23 *5.3*	33 7.6
.1	384	15[a] 3.9[b]	44 11.5	114 29.7	128 33.2	29 7.6	20 5.2	21 5.5	13 3.4	0 0	34 *8.9*	54 14.1
Unsprayed	314	0[a] 0[b]	4 1.2	0 0	29 9.2	0 0	26 8.2	184 58.7	56 17.9	15 4.8	255 *81.4*	281 89.6

[a] Volumes given on this line.
[b] Percentages of different grades given on this line.

fairly good reduction of round- and flat-headed borer damage but no control of ambrosia beetle damage) compared much more closely with downgrading in the 0.2 and 0.4% gamma isomer treatments (where good prevention of all types of insect damage was achieved) than it compared with downgrading in the unsprayed check logs (where all types of insects were abundant).

The percentages of lumber downgraded to Number 5 common because of other causes (wane and decay) was only 0, 2.3, 5.2, and 8.2% of the lumber from the logs sprayed with 0.4, 0.2, and 0.1% gamma isomer and from the unsprayed checks, respectively (table 2).

It should be noted in table 2 that much higher percentages of the better grades of lumber (D Select and better, down through Numbers 1, 2, 3, and 4 common) were sawed from the three groups of sprayed logs than from the unsprayed checks. Without this downgrading due to worm holes, the unsprayed check logs would have produced a grade yield of lumber that was much more comparable with that produced by the sprayed logs. There would, of course, be variations in the grades of lumber sawed from individual logs and from the four groups of logs based on factors and defects other than those caused by insects.

Although no records were kept of stain in lumber that was downgraded to Number 5 common, generally speaking, stain seemed most abundant in Number 5 common boards; a vast majority of which was sawed from the unsprayed check logs where insect damage was most abundant. This observation seems quite in line with the findings of McNeel *et al.* (1952) who, as a result of spraying tests with Jack pine logs in Wisconsin, reported, "Blue stain and decay are definitely associated with insects, and consequently fungal development is retarded by insect control."

SUMMARY.—Based on examination of the sapwood surface of the logs in the autumn, single early spring applications of lindane emulsion sprays containing 0.4 or 0.2% gamma isomer (by volume) to individual white pine sawlogs gave 87.2 to 100% protection against all insect damage through the entire summer. A 0.1% gamma emulsion gave 85.1 to 99.3% protection against all insects except ambrosia beetles, against which it apparently failed.

In grading the lumber sawed from all the test logs according to the standard grading rules of the Northeastern Lumber Manufacturer's Association, downgrading one or more grades to Number 5 common because of insect damage in the 0.4, 0.2, and 0.1% gamma isomer treatments amounted to 6.1, 5.3, and 8.9%, respectively, compared with 81.4% in the unsprayed checks. This downgrading was attributed chiefly to large worm holes made by round- and possibly some flat-headed borers. Additional downgrading due to the tiny pinworm holes made by ambrosia beetles was not significant in this test.

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The Pollination of Hairy Vetch by Honey Bees[1]

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Insect pollination is necessary for maximum seed or fruit yields from a large number of agricultural crops. In many areas intensive agriculture and the widespread use of insecticides have so reduced the population of indigenous pollinators that honey bees have assumed increased importance in the production of seeds and fruits.

MATERIALS AND METHODS.—Experiments were conducted on Crocket fine sandy clay loam soil near Terrell, Kaufman County, Texas, in 1953, 1954 and 1955 to determine the value of honey bees (*Apis mellifera* L.) and other insect pollinators to seed production by hairy vetch (*Vica villosa* Roth.), and the value of vetch as a source of nectar and pollen for bees. Seventh year volunteer vetch which had received 125 to 175 pounds per acre of superphosphate, 1-14-7, or 3-12-12 fertilizer annually was used in the experiments in 1953. In 1955 the experiment was again conducted in the field used in 1953, but the field had been virtually without vetch in 1954, and was reseeded in the fall of the year. The experiment in 1954 was conducted in a field of first year vetch which had been in pasture for 20 years previously. The seed bed was treated with 160 pounds per acre of 3-12-12 fertilizer containing trace elements, and planted with 18 pounds per acre of vetch seed in the fall of 1953.

Destructive insects in and around the experimental plots in 1953 were controlled with 2 pounds of toxaphene per acre in a spray on May 1, $\frac{1}{8}$ pound of demeton per acre in a spray on May 26, and 10 pounds per acre of 20% toxaphene plus 40% sulfur dust on June 1. In 1954, 2 pounds of toxaphene plus $\frac{1}{4}$ pound of demeton per acre was sprayed on the vetch on April 20, 10 pounds per acre of 20% toxaphene plus 40% sulfur dust was applied on May 4, 14, and 21, and 2 pounds of toxaphene plus $\frac{1}{16}$ pound of demeton per acre was sprayed on the vetch on May 26. In 1955, 2 pounds of toxaphene plus $\frac{1}{4}$ pound of demeton per acre was applied on April 14, and 10 pounds of 20% toxaphene plus 40% sulfur dust per acre was applied on April 28 and May 17.

The longevity and characteristics of vetch blossoms

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were determined by tagging individual racemes of buds in uncaged plots and in plots caged to exclude bees, and making observations on the racemes at least once daily. The amount of bloom was estimated weekly from the number of open, unwithered blossoms in 1 square yard of vetch without skips between the vines. These areas were not randomly selected, but since there was a good stand of vetch in the experimental area, they gave fair approximations of the bloom in that part of the field.

The nectar concentration was determined by examining the contents of the honey stomachs of bees in the field. A bee was captured, her honey stomach opened, and the percentage of total solids in the nectar was read directly to the nearest 1% on a Bausch and Lomb or Zeiss hand refractometer. To minimize evaporation, only one bee was used per reading and all operations were performed as quickly as possible. The mean of at least two uncorrected readings was considered to be the sugar concentration in the nectar at any given time. Data taken between 1:00 and 4:00 P.M. were used to study the daily fluctuations in nectar concentration.

The honey flow was measured by taking the gain in weight from one morning to the next of a strong colony of bees on scales. Daily weights were taken when possible; additional weighings were usually made at hourly intervals during the day if the bees were foraging. The contents of a pollen trap were used to measure the amount and percentage of vetch pollen gathered by the bees. The plants from which individual pollen loads came were determined by the method of Maurizio (1953) and by color.

In 1955 the amount of nectar available from blossoms was measured by a method similar to that described by Swanson & Shuel (1950). Vetch was protected from bee visits by caging for 1 to 4 days, and then racemes were removed from the cage, the buds, withered blossoms, and partially withered blossoms were removed from the racemes, the terminal half of each blossom was cut off, and four of the racemes were centrifuged for 5 minutes in an International Clinical centrifuge at 3200 r.p.m. During a part of the season four racemes from uncaged vetch were similarly centrifuged. All measurements were made between 1:00 and 2:00 P.M. from racemes containing at least 15 open, unwithered blossoms each.

The number of foragers in the field was estimated from data obtained by staking off a 1-square-yard area in each uncaged plot, and counting the number of foragers in the areas on different days and at different times of the day. Counts were made only when there was flight from nearby colonies of bees. For estimates of the number of foragers in other parts of the field or in other fields, the number of bees foraging in successive estimated 1-square-yard areas was used. Details of the methods used in studies of foraging behavior are given elsewhere (Weaver 1956).

Plastic screen cages similar to those described by Pederson *et al.* (1950), but with galvanized iron pipe frameworks 6×6×36 feet were constructed over 10 randomly selected plots in the experimental area in 1953 and 1955. Small colonies of bees were placed in five of the cages, and the other five cages excluded bees and other pollinating insects. Five additional plots of the same size were left uncaged so that bees and other insects had free access to them. In 1954, the experimental design was the same, but there were five additional randomized caged plots in which colonies of bees were placed for 1 day each week, and which were without pollinators on the remaining days. In 1953 and 1954 the vetch in the plots was disturbed as little as possible after the cages were constructed, but in constructing the cages and applying insecticides there was some trampling of the vetch around the edges of each plot. This damaged vetch appeared to interfere with the growth and vigor of the adjacent vetch plants, so in 1955 after the cages were constructed, a strip of vetch slightly under a foot wide was removed from the outer edge of each plot. Two wires about 2 feet high and 3 feet apart were then strectched longitudinally in each cage, but not in the open plots.

When the seed pods were mature, a randomly selected 1-square-yard area in each plot was clipped by hand, bagged, air dried, and the seed pods were picked and thrashed by hand. They were cleaned in an air blower and graded by use of hand screens and an inclined screw-type seed separator. Every effort was made to prevent the loss of seed during these operations. The No. 1 seeds were weighed to the nearest 0.1 grams and converted to pounds of seed per acre.

The number of seed in 100 randomly selected pods from each plot and the weight of 100 randomly selected No. 1 seed from each plot were recorded. All of the pods or seed from a plot were used for these measurements if there were less than 100 available from the plot. Germination tests were conducted on the No. 1 seed produced in 1955. Four replicates of 25 seed from each plot were held between damp blotters at 68° F. for 14 days and the number of germinated seed and hard seed was recorded.

Data on seed yield, number of seed in pods, weight of seed, and germination of seed were subjected to analyses of variance by the methods of Snedecor (1946).

RESULTS.—It is not known how long vetch blossoms are susceptible to successful pollination, but presumably they will develop seed after successful pollination during most of the time that they are open and unwithered. The anthers of hiary vetch dehisce before the blossom is completely open. During cool weather the more vigorous unpollinated blossoms remain open for 4 to 6 days; all the blossoms on a few racemes and a few blossoms, especially the terminal ones, on most racemes wither or are shed in 1 to 4 days; during hot weather unpollinated blossoms usually wither within 2 days. Within a few hours after being pollinated a blossom begins to wither, but if seed are set the withered blossom clings to the raceme until growth of the developing pod bursts it.

In many regions of the state, vetch is considered a very poor source of nectar, but in the region in which these experiments were conducted it was, at its best, a very good nectar source, as can be seen by an examination of figure 1, which shows the daily change in weight of strong colonies of bees foraging from vetch in 1953, 1954, and 1955 when it was the predominant nectar source all 3 years. Figure 1 also shows the hourly change in weight to the nearest $\frac{1}{4}$ lb. of the colony on scales on May 15, 1954. The colony gained almost 11 pounds of nectar during the day, and by the following morning had lost only 3 pounds by evaporation of water from the nectar and other causes. It will be noted that the colony lost 5 pounds

between 7:00 and 9:00 A.M.; most of this loss was caused by foraging bees going into the field. During the fast nectar flows the colonies never lost more than 2 pounds in the morning, doubtless because some bees returned to the hive with nectar before others went into the field, but similar losses were common on days when the bees stored about 10 pounds of nectar.

In 1953 the experimental field was among the last in the area to come into bloom, and although there was an apiary in the field, there were few bees working the vetch until a colony on scales was moved into the field. At that time the vetch was approaching full bloom, and from then until bad weather intervened there were 0.5 foraging bees per square yard of vetch. As can be seen from an examination of figure 1, the colony gained 102 pounds in 6 days. It is probable that inadequate storage space and some rain on the last 2 days kept the gain from being greater. During the 4 best days colonies with about 3 pounds of bees in the 210 square foot cages stored surplus nectar, and the nectar concentration remained near 60% sugar. Following a week during which rain fell every day, however, diseases attacked the vetch and there was little further bloom until about May 22. By then the vetch had put on new growth and for about a week there was an extremely heavy bloom, which reached a peak of more than 7,000 blossoms per square yard, but virtually all the blossoms were gone by June 1. During the period when there was little bloom many of the surrounding vetch fields were cut for hay, and there were 1.8 foragers

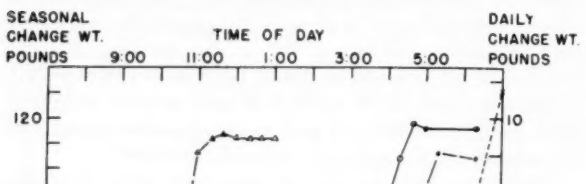

FIG. 1.—Daily changes in weight of colonies of honey bees foraging from hairy vetch in 1953, 1954 and 1955, and hourly changes in weight during May 14, 1954.

per square yard in the experimental field during the late season bloom. The nectar concentration ranged from 36 to 49% sugar during this time, and the colony gained about 28 pounds during the 6 days when suplus nectar was stored.

The bloom, nectar concentration, nectar yield, bee population, location of apiaries, and the amount of bloom in comparison with surrounding fields were similar early in 1953 and 1955. There were 0.6 bees per square yard during the first week of bloom after a scale colony had been moved into the field in 1955, and the colony gained 113 pounds in 7 days. There was up to 1.2 μl. of nectar per blossom, and a mean of 0.8 μl. per blossom in the caged vetch during those days. A moderate amount of disease attacked the vetch during several days of cloudy weather with intermittent showers. Many of the bees from the nearby apiary evidently changed foraging areas during this cloudy weather, and there was a mean of 2.7 bees per square yard during the remainder of the blooming period, with means of over four bees per square yard being common during periods of maximum foraging activity. Since both maturing seed pods and disease reduced the bloom, the vetch was badly over-crowded with foragers, and the colony lost more weight than it gained. The caged vetch still yielded a mean of 0.5 μl. of nectar per blossom, but uncaged vetch yielded a mean of 0.1 μl. per blossom. After about a week only scattered bloom remained, though some of the plants continued to put on new growth which bloomed until the last of May.

In 1954 the experimental field was among the first in the area to come into bloom. Diseases attacked the vetch during the two rainy periods in late April; seed pods and leaves were continuously being shed as disease progressed up the stems, but the terminals continued to grow and bloom vigorously. From April 26 through May 27 the amount of bloom was almost constant at about 4,600 open, unwithered blossoms per square yard. Though the nearest apiary was about $\frac{1}{4}$ mile away there was a mean of 2.2 foraging bees per square yard of vetch during 9 days early in the season when the weather allowed normal foraging. The colony on scales gained about 52 pounds during those days. After 4 days of inclement weather about the middle of April, many of the foragers deserted the field, and a mean of 1.1 bees per square yard remained during the rest of the season. During the 12 days that the weather permitted approximately normal foraging and there was a nectar flow, the colony gained over 76 pounds. The nectar concentration fluctuated more sharply in 1954 than in the other years, but it seldom reached 50% sugar.

Bees foraged older blossoms in preference to newly opened ones, and these undoubtedly contained more nectar than is indicated by the data on nectar yield from caged blossoms, since the means were based on racemes containing both old and young blossoms. The data on blossoms taken from the open field late in the season, however, may overestimate the amount of nectar per blossom, since racemes with more than 15 open, unwithered blossoms were selected, and these were rare. The blossoms that had been tripped withered quickly, and limited measurements indicated that they contained little nectar. Usually only a tiny droplet of nectar is visible on the nectary, but in 1953 it was observed that a

few of the corolla tubes were filled with nectar to a depth of about 1 mm. A similar accumulation of nectar in blossoms has been observed in unforaged crimson clover (Weaver & Ford 1952). In 1955 the accumulation of nectar was not this great, though a film of nectar sometimes covered the bottom of older blossoms. The experimental field, early in 1955, had nectar of a higher sugar concentration and much more nectar per blossom than at least one nearby field that had come into bloom earlier, and that had more foragers. This probably made it easier for scout bees to recruit foragers previously attached to another area. In 1955 both the interruption of foraging and the threat of rain which made it dangerous for the bees to go far from the hive may have contributed to the change in foraging areas, but in 1954 the change occurred after several days without the threat of rain, but with cold weather which interrupted foraging.

The high concentration of bees in the field early in 1954 and late in 1953 and 1955 may have contributed to the poor nectar income to the colonies during those times. It would also account for the small amount of nectar in blossoms unprotected from bees late in 1955, when the amount of nectar in caged blossoms was about one-half as great as it was during the best honey flow. Prolonged and detailed studies will be necessary to determine the number of bees that can be profitably kept for honey production on a given area of vetch under different conditions.

The factors that affect the amount and concentration of nectar secreted by any plant are not well understood (Beutler 1953). The concentration varies inversely, to a certain extent, with the relative humidity. On days when the air was humid in the morning and dry in the afternoon, the sugar concentration of vetch nectar sometimes increased more than 30% during the day, so it is important to take into account the weather conditions at the time a determination is made. From all the data that were gathered it appears likely that vigorous vetch that has just started to bloom and that is not growing rapidly yields the most nectar with the highest sugar content. The amount of nectar and its sugar concentration seemed to be reduced when vetch was heavily diseased, was putting on heavy new growth, or was maturing many seed.

During the 3 years these experiments were conducted, diseases of vetch probably limited seed production more than any other single factor except lack of pollination. Most of the variation between plots receiving the same amount of pollination was caused by variation in the severity of the diseases. Vetch supported by a nurse crop of grain, or by wires, had less disease than unsupported vetch; the diseases were more severe inside the cages than outside; caged plots between two other cages had more disease than those adjacent to open plots; and the open plots interspersed between cages had more disease than the surrounding field. According to Whitehead's (1954) determinations, species of *Colletotrichum*, *Fusarium*, *Alternaria*, *Hormodendron*, *Helminthosporium*, *Erysiphe*, *Trichoderma*, and *Ascochyta* were the main causative organisms. All of the seed set early in 1953 and 1954 were shed as the disease progressed up the stem, and during those 2 years most of the seed that matured was set during the last week or two of bloom. In 1955 a heavy seed crop was set before the vetch became moderately diseased, and observations indicated that about half of the pods were shed, or failed to develop No. 1 seed after diseases attacked the vetch.

In 1953 colonies containing about 3 pounds of bees were moved into the caged bee plots. There was such an excess of foraging bees in the small area that flower buds and blossoms were visited repeatedly for both pollen and nectar, and the anthers, stigmas, styles and corollas were usually badly damaged. An excess of pollen in the hive did not noticeably affect foraging behavior, and did not stop the bees from damaging the blossoms. When most of the bees were removed from the colonies so that about ½ pound of bees in 3-frame nuclei remained, few blossoms were damaged. It is believed that the instinct to forage and the insufficient number of blossoms for normal foraging, rather than the direct effects of caging on the colonies or on the individual bees, led to the damage to blossoms. It is essential that colonies of bees in cages be supplied with sufficient amounts of pollen. Colonies deprived of pollen for more than about 2 weeks quit behaving as normal colonies and finally cease normal foraging.

Table 1 gives the yield of No. 1 vetch seed, from each plot, converted to pounds of seed per acre. The F value in all experiments was highly significant, and most of the variation between treatments was caused by the failure of unpollinated vetch to yield much seed. Since the only known difference in the plots caged with bees and those caged without bees was in the level of pollination, the 10-fold or greater increase in seed yield in the caged bee plots can be attributed to pollination by honey bees. The cages which contained bees 1 day each week in 1954 and excluded them on the remaining days supplied a third level of pollination. The fairly high seed yield in these intermittently pollinated plots indicates that continuous pollination is not necessary for good vetch seed yields. Healthy vetch would doubtless have given a better understanding of the value of intensive intermittent pollination. The differences in plots caged with bees and uncaged plots reflect the level of pollination, the effects of caging on vetch, and, in 1955, the effect of partially supporting the vetch on wires. The severe disease in the caged bee plots apparently limited seed production there, and it is believed that in open plots the

Table 1.—Pounds of No. 1 hairy vetch seed per acre from plots with pollination as shown.

Year and Pollination	Pounds of Vetch Seed per Acre						F Value
	In Plots					Mean	
1953							
Caged with bees	643	322	502	446	238	430	
Caged without bees	6	18	17	31	20	18	
No cage	389	309	613	561	411	457	22.2[a]
1954							
Caged with bees	313	276	378	191	145	261	
Caged without bees	2	2	<1	2	<1	1	
Intermittent caged bees	635	109	79	149	238	242	
No cage	366	406	436	153	394	351	6.1[a]
1955							
Caged with bees	509	881	583	466	669	622	
Caged without bees	52	44	55	59	46	51	
No cage	319	646	448	374	376	433	29.21[a]

[a] Probability <0.01.

number of bees was sufficiently large to pollinate more blossoms than the vetch plants could have matured under the best conditions except early in 1953 and 1955. No seed matured from the blossoms pollinated early in 1953, but in 1955 many early pods did mature and there were more seed and far more flat pods in the plots caged with bees than in the uncaged plots.

In 1953 there was a mean of 179 seed per 100 pods in the plots caged to exclude bees, 335 seed per 100 pods in the plots caged with bees, and 340 seed per 100 pods in the uncaged plots. The differences were highly significant. Similar results were obtained in 1954 and 1955. The mean weight of 100 seed produced in the plots caged without bees in 1953 was 2.8 gm., from the plots caged with bees, 1.8 gm., and from the open plots, 1.9 gm. These differences were highly significant, and the results were verified the following 2 years. It is probable that heavier seed developed in the cages without bees because more nutrients were available per seed from vines with fewer developing seed. There was no difference due to treatments in the percentage germination of the seed.

In addition to bearing heavier seed, the unpollinated vetch also continued in heavy bloom for 1 to 3 weeks after the bloom was virtually gone from the vetch in the cages with bees, and the total number of blossoms each season was doubtless greater in the unpollinated than in the polinated vetch. This was particularly true in 1955 when most of the pods in the unpollinated plots had one or more good seed in them and observations indicated that few pods that began development in those plots were shed before the harvest. From these considerations, and from the seed yields, it is obvious that vetch is highly self sterile, and, since the seed that developed in these plots could have resulted from cross pollination by insects that live in vetch, the extent of the self sterility is not known. Thrips, moths, and butterflies were fairly numerous in the caged plots in 1953 and 1955, so these insects are of negligible value as pollinators. It is possible that better control of these insects, however, helped to cause the virtual absence of seed in the plots caged without bees in 1954.

Several species of wild bees are efficient pollinators of vetch, but the region in which these experiments were conducted is intensively cultivated, and wild bees are virtually absent. A few individuals representing 1 species of carpenter bee, 1 species of bumblebee, and 2 species of Megachilidae were seen during the 3 years. Of these, the carpenter bees punctured the corolla tube rather than tripping the blossom. Honey bees were the only effective pollimators of vetch numerous enough in the area to be of any real value to seed production.

The mature seed from hairy vetch, even if it has maximum pollination, represents only a small fraction of the potential production. There are seven ovarioles per ovary and a mean of 25 blossoms develop on each raceme. On one day during the heaviest bloom in 1953 there were 691 racemes with over 7,400 open, unwithered blossoms in 1 square yard of vetch. There was an average of about 4,600 open, unwithered blossoms on 550 racemes according to 5 weekly counts in 1954. In vetch with an adequate number of pollinators many tripped blossoms are shed before seed development becomes noticeable. This could be caused by insect attacks, by morphological imperfections or the physiological condition of the vetch plant, by inadequate cross pollination, or by cross pollination with a mutually sterile plant. In crimson clover it was observed that bees pushed the head straight into the corolla tube to reach the nectar, and that the posterior side of the head became covered with pollen (Weaver & Ford 1953). The prolonged and intimate contact with the sexual column afforded good opportunity for cross pollination by nectar gathering bees. In vetch there is often only slight contact between the sexual column and the head or thorax of the nectar-gathering bee. When visiting blossoms that had previously been tripped, bees often made no contact with the antlers and stigma. Pollen gathering bees contact both the stigma and anthers more than nectar gatherers, so it seems probable that the highest percentage of cross pollinated blossoms would be attained by a pollen collecting bee which visited only one blossom on each vine. Studies indicate that bees work an average of about two blossoms per raceme and that they move more than 4 inches between racemes only about 50% of the time (Weaver 1956). Since vetch plants intertwine, it is impossible to know how often bees move from one plant to another.

In order to estimate the number of honey bees necessary to pollinate a field of vetch, the population of indigenous pollinators as well as several other factors must be taken into account. One of these factors is the amount of competition from other plant species or other fields of vetch for the bees' visits. In some regions competition from other species can be severe, especially if the vetch is a poor source of nectar. In the region in which these experiments were conducted vetch was virtually the sole source of pollen and nectar while it was in bloom; more than 90% of the pollen caught in a pollen trap was from vetch. Even where there is no competition from other plants, the amount of nectar secreted by the vetch will affect the number of honey bees necessary for adequate pollination. Bees are able to reach the nectary without tripping the blossom by inserting the tongue between the petals of the standard and the keel at the base of the corolla tube. These non-tripping bees foraged faster than the trippers and when the nectar flow deteriorated, the proportion of non-trippers increased. Although adequate data were not gathered for vetch in all conditions, it appeared that there were fewer foragers and that a larger percentage of the foraging bees were non-trippers in vetch lacking in vigor, and presumably secreting less nectar (Weaver 1956).

DISCUSSION.—From data and observations on the blooming characteristics of vetch and on the foraging behavior of bees, it is possible to make a rough calculation of the number of bees necessary to adequately pollinate vigorous vetch which has no indigenous pollinators, and no competition from other plants, for bee visits. One bee tripping 3.6 blossoms per minute (Weaver 1956) for 10 hours should be able to visit the blossoms that open on 1 day in 1 square yard of vetch. In practice some blossoms would be visited repeatedly and others would not be visited at all with this level of pollination. Even among the tripped blossoms there is no way of knowing how many are effectively cross pollinated, so in spite of a fairly large safety factor, the calculated one bee per square yard may be too small for maximum pollination. A higher

concentration of bees would also be advantageous by causing the bees to fly greater distances more often (Weaver 1956) and thus would favor more cross pollination. From these considerations, and from further calculations, some of them based on estimates, it would appear that at least one strong colony of bees would be necessary to supply maximum pollination for an acre of vetch. In practice, however, maximum pollination may not be economical. Even in ideal seasons and with ideal vetch the return in mature seed probably would not be increased greatly by an increase in the number of pollinators beyond the number required to trip a majority of the blossoms. The amount of honey stored per colony would be decreased by such an increase in the number of bees. It is estimated that one strong colony of bees should supply adequate pollination for 3 to 5 acres of good vetch. At this level of pollination the honey crop usually should pay for the pollination services in regions where vetch is a good source of nectar, and in regions where it is not, the seed grower usually should be able to pay for the pollination service from the increase in seed yield. These are provisional estimates which may have to be modified when more data have been obtained on the seed and nectar yield from vetch with known populations of pollinators.

SUMMARY AND CONCLUSIONS.—Observations were made on the blooming period, nectar yield and concentration, and pollination of hairy vetch in 1953, 1954 and 1955, and on the population of foraging bees and some aspects of their behavior.

Each year diseases of the vetch drastically affected the blooming period, nectar flow, and seed yield.

More work is needed on the factors that affect nectar yield by vetch plants, but the secretion appears to be greatest early in the season if the vetch is not putting on much new growth, and poorest when the vetch is growing rapidly, is heavily diseased, or is maturing much seed.

Vetch was a good but erratic source of nectar in the region in which these experiments were conducted, providing it was not overcrowded with bees.

Vetch was virtually the only source of nectar and pollen, and honey bees were virtually the only pollinators of vetch during these experiments.

Bees tend to continue to forage in the area they first visit, and an interruption of foraging seems to make it more likely that they will change to an area with richer forage.

Experiments with uncaged plots, and with plots caged to enclose or exclude bees, showed that vetch with adequate numbers of pollinators yielded more than 10 times as much seed as unpollinated vetch. There were fewer seed per pod, but heavier seed, in unpollinated than in pollinated vetch; there was no difference in the germination of such seed.

It is tentatively concluded that one strong colony of bees should be able to pollinate for 3 to 5 acres of vetch adequately, but at least one colony would be needed for maximum pollination of an acre of vetch. This subject needs more study.

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Length of Larval Period Related to DDT-Resistance in *Drosophila*[1,2]

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This paper is one of a series on the genetics and ethology of DDT-resistance in *Drosophila* and house flies conducted at the Department of Entomology of the University of Kansas.

Phenotypic differences between DDT-resistant and non-resistant strains of flies should be expected if the genes causing resistance also affect other measurable characters directly by pleiotropism, or indirectly by linkage with genes controlling other morphological or physiological traits. Sokal & Hunter (1954) found that pupation site reflected the DDT-resistance level of larvae of each of five *Drosophila* strains.

Fecundity, fertility, developmental time, weight of different life stages, as well as some 20 or more morphological characters have been studied in a search for correlates of resistance in house flies. Of these characters we shall consider length of developmental stage only. Bruce (1949) reported that resistant house fly strains required 1

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to 2 days longer for development than a control strain in his laboratory. Pimental *et al.* (1951), using four resistant house fly strains, have shown close correlation between length of the larval period and DDT-resistance; the larval periods of all four resistant strains were significantly longer than those of a non-resistant control strain. McKenzie & Hoskins (1954) selected for early and late pupation of house flies and found that the strain selected for late pupation was more resistant to DDT and chemically related insecticides than the strain selected for early pupation. However, further selection for late pupation in the late pupating strain led to a simultaneous decrease in length of larval period and resistance so that the strain eventually became more susceptible than the control strain. Johnston *et al.* (1954) found that when the developmental period of a non-resistant house fly strain was lengthened by lowering the rearing temperature, there was an increase in the DDT-resistance of the adults from these cultures.

A reverse situation was noted by Gagliani (1952). Using flies resistant to both DDT and chlordane, Gagliani found that the resistant flies had an average larval period which was 12 hours shorter in duration than the average larval period of a laboratory strain.

In contrast to the above findings, March & Lewallen (1950) reported no significant difference in length of the larval period between the resistant and non-resistant house fly strains they studied. Similar results were found by Babers *et al.* (1953), who reported a large variation in the lengths of larval period among the eight strains used in their tests, but found no consistent differences between resistant and non-resistant strains.

These investigations show that longer larval period does not accompany DDT-resistance in all cases. The absence of such a correlation might be explained by the occurrence of different genetic systems causing DDT-resistance. Such different systems have been demonstrated in house flies (Maelzer & Kirk 1953) and *Drosophila* (King 1954); thus resistance may be correlated with, or even caused by, a physiological or morphological character in one case and not so related in a second case owing to different genetic factors.

Correlation between resistance and length of the immature stage in house flies has been observed often enough to warrant a search for this phenomenon in other diptera. Sokal (personal communication) has been unable to find consistent differences in length of the larval or pupal period among various *Drosophila* strains differing in larval resistance to DDT. However, it seemed worth while to investigate the reverse situation and determine if selection for length of larval period would result in changes in DDT-resistance.

MATERIAL AND METHODS.—Two way mass selection of *Drosophila melanogaster* has been carried out since 1952. One line, referred to as LL, is being selected for long larval period while the other line, SL, is being selected for short larval period. An unselected line, referred to as CS, has been maintained as a control. These lines are in the 84th generation of selection at the time of writing (January, 1956). The original stock, designated as COSU-1, which has served as a foundation strain for a number of other selected lines in this laboratory, was produced by three generations of special crosses, including reciprocal crosses, of four strains. These strains were the Canton-S, Oregon-R-C, Sweden-C, and Urbana-S, which were maintained at the Genetic Laboratory of the California Institute of Technology for a number of years prior to the discovery of DDT. When selection for the present experiment was initiated, this stock had been maintained by mass mating for six generations.

In each strain[4] selection intensities varied with the population size of each particular generation. Approximately 22 parent pairs were taken from the tail of a distribution of 200 flies or less in the selected strains. Two-hundred eggs were taken at random from an oviposition bottle containing the parent flies, and offspring from these eggs were reared to produce the next generation. The control flies were reared in the same manner as the selected strains except that parents for ensuing generations were chosen at random.

Flies were reared singly in 1-dram shell vials (size: 15×45 mm.). Each vial was filled with a corn meal-molasses-agar medium to a height of approximately 10 mm. The vials were numbered, and a record was kept of the time of hatching and pupation of the fly in each vial, making it possible to determine the length of the larval period of each individual.

Although during the earlier generations of selection the differences in length of larval period between the selected strains fluctuated considerably, these differences became more or less constant by the 28th generation. At this time the larval period of the LL strain was 8 hours longer on the average than that of the CS strain; whereas the SL strain had a larval period of about 10 hours less than that of the CS strain, adding up to a difference of approximately 18 to 20 hours in length of larval life between the two selected strains.

At various generations of selection the larvae were assayed for DDT-resistance by adding known amounts of *p, p*-DDT dissolved in carbon tetrachloride to liquid larval medium to produce eight different concentrations of DDT ranging from 3.4 to 9.8 p.p.m. The carbon tetrachloride evaporated from the medium and left no demonstrable toxic residue in control experiments. In these assay tests the flies were reared, 10 per vial, in 6-dram shell vials (23×85 mm.) filled to a height of 15 mm. with larval medium. Approximately 18 such vials were used for each DDT dose. The total mortality—including larval, pupal, and adult mortalities—was recorded for each vial. This method of DDT assay is standard procedure in this laboratory.

RESULTS.—Generations 29, 30, 40, 47, and 53 were assayed for DDT-resistance. Probit regression lines were fitted by the method of maximum likelihood (Finney 1952) to each strain for each of the generations tested, and the results are shown in table 1. In all tests, except those of generation 30, the SL strain has the steepest slope (larger regression coefficient—*b*), the LL strain is intermediate between SL and CS, and the CS strain has the shallowest slope. In generation 30 the CS strain has the steepest slope, while the LL strain has the shallowest.

"t" tests for parallelism of the dosage-mortality regression lines for the three strains gave the following results:

[4] Although "lines" is the accepted term for closed populations subjected to selection, "strains" will be used in the rest of this paper to avoid confusion with regression lines mentioned repeatedly below.

Table 1.—Regression coefficients (b) with standard errors and pertinent degrees of freedom (identical for strains within generations) for various generations of selection and for the pooled data of these generations.

Generation Number	Degrees of Freedom	Strains		
		SL	CS	LL
29	3	7.1±1.28		2.7±0.50
30	4	5.3±1.05	6.0±0.45	4.3±0.48
40	4	5.3±0.96	2.1±0.42	4.9±0.52
47	5	3.4±1.10	1.8±0.33	2.3±0.40
53	2	5.3±0.55	3.1±0.49	4.0±0.47
Pooled data	6	4.6±0.19	3.1±0.18	3.1±0.19

only in generation 40 was the deviation from parallelism significant when the SL and CS strains were compared; SL tested against LL showed a significant t value in generation 29 only; CS tested against LL showed significant differences from parallelism in generations 30 and 40. The larger standard error of b in the SL strain is due to correction for heterogeneity necessary in that strain. Only in generation 53 was no correction needed.

Table 2 shows that in each generation tested the LD_{50} of the SL strain was lower than that of the LL strain. However, only in generations 30 and 47 were the fiducial limits of one selected strain beyond the limits of the other selected strain, and only at generation 30 was there no overlapping among the fiducial limits of the three strains. In general, the CS strain was equal to or slightly more resistant than the LL strain.

The LD_{50} values and the extent of the fiducial limits varied from generation to generation without any apparent trend. This variation is inherent to our method of assaying resistance and is probably due to environmental differences. Much of the difference in the extent of the fiducial limits from generation to generation, and among selected strains within generations, is due to the presence of heterogeneity in the data. Correction for heterogeneity resulted in a wider range of the fiducial limits.

Since the regression lines for the selected and control strains were not parallel, it was not possible, in this analysis, to determine the relative resistance of the SL strain compared to either of the other strains.

The bottom line of table 2 shows the results of pooling the data for all generations and treating these data as a single test. Uncontrolled variation of the single generations apparently occurred in a random fashion, and the differences of one generation appear to cancel out those

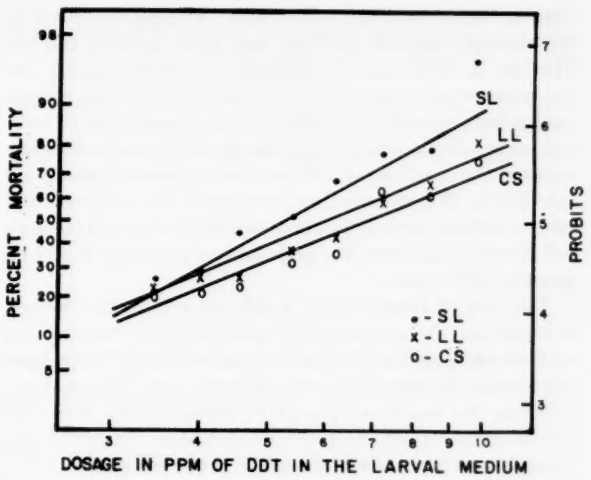

Fig. 1.—Dosage mortality regression lines fitted by maximum likelihood for the three strains for pooled data of generations 29, 30, 40, 47, and 53.

of another. As a result no heterogeneity appears in the pooled data.

Analysis of the pooled data by the maximum likelihood method showed that the regression line for the SL strain was significantly different in slope from either the LL or CS strains; however, no significant difference in slope existed between the LL and CS strains (Fig. 1).

Fiducial limits ($P=.05$) set to the LD_{50} values of the pooled data show that the SL strain has a significantly lower level of DDT tolerance than either the LL or CS strains. It was not possible to demonstrate any difference in DDT-resistance between the LL and CS strains. Only by forcing parallel regression lines upon the data would it be possible to calculate the relative resistance of any two strains. When this was done, no difference in DDT-resistance could be shown between any of the three strains. By forcing parallelism upon the regression lines the variance of the points around the regression line is increased, and only large differences in the original data can be demonstrated by such statistical treatment.

Although the difference in DDT-resistance involved in this work is not large, it is in keeping with the results of most DDT-resistance work in *Drosophila*, i.e. resistant strains rarely become more than a few times more resistant than they were originally.

Discussion.—The inadvisability of interpreting re-

Table 2.—LD_{50}'s (in p.p.m. DDT) and their 5% fiducial limits for various generations of selection and for pooled data of these generations.

Generation Number	SL Strain			CS Strain			LL Strain		
	Lower Limit of LD_{50}	LD_{50}	Upper Limit of LD_{50}	Lower Limit of LD_{50}	LD_{50}	Upper Limit of LD_{50}	Lower Limit of LD_{50}	LD_{50}	Upper Limit of LD_{50}
29	4.3	5.0	5.9				5.3	5.8	6.6
30	3.7	4.1	4.6	5.1	5.3	5.5	5.5	5.9	6.3
40	6.3	6.8	7.4	8.4	9.2	10.2	6.9	7.5	8.1
47	2.1	3.1	4.5	5.1	5.8	6.4	4.6	5.1	5.6
53	5.3	5.6	6.0	5.7	6.3	7.0	5.8	6.2	6.7
Pooled data	4.9	5.0	5.1	6.3	6.6	6.9	6.1	6.3	6.5

sistance based on LD_{50} values only, without reference to the dosage response pattern, has been pointed out by Maelzer & Kirk (1954). Complete information on the dosage-mortality regression lines will reveal two important points, namely: (1) differences in the amount of toxicant necessary to produce a given mortality and (2) differences in the reactions of different test strains to a common insecticide. When strains are compared, the occurrence of one or both of these differences would indicate physiological diversity between the populations probably based on genetic differences.

The first of these points, a difference in lethal dosage, is important from a practical point of view since it tells us that more or less toxicant is required to kill the same proportion of animals in two different populations. Considering the results of the DDT assays given above, it can be stated that less DDT is needed in the larval medium to produce a 50% mortality in the SL strain of flies than for either the CS or LL strains. On the other hand, selection in the LL strain of flies produced essentially no change in DDT-resistance over that of the control strain.

The second point mentioned above refers to differences in the slopes of the regression lines. These differences may also be important from a practical standpoint since equal increase in DDT dosage may not produce an equal increase in mortality in populations having different regression slopes. It is probable that unequal slopes reflect genetic differences between strains. Non-parallelism in the regression lines would indicate that the tolerances of the populations tested have different variances and probably different distributions.

The steeper slope of the SL regression line indicates less variation in DDT-resistance within this strain compared to the CS or LL strains. This increased homogeneity of the SL strain is also shown in the data on length of larval period where the variance of the SL strain is always less than the variance of the LL strain and generally less than the variance of the CS strain. This lowering of the variance of the SL strain is quite likely due to a decrease of the genetic variance of the characters concerned.

Selection of the SL strain has resulted in the loss of some of its original ability to withstand DDT poisoning. The exact processes involved are not known. In house flies, Sternburg *et al.* (1954) have shown that all adult flies resistant to DDT have a DDT-dehydrochlorinase enzyme, which metabolizes DDT to the non-toxic DDE. This enzyme is apparently absent, or present in only very small amounts, in susceptible house flies. Perry & Sacktor (1955) have concluded that adult resistance may be produced by several different factors which are not necessarily the same in all strains; thus, different strains may show DDT-resistance due to different causes.

Some probable reasons for the loss of resistance in the SL strain may be mentioned. Since the SL flies require less time for larval development than the CS or LL strains, an increase in the metabolic rate of the SL flies seems possible. If such an increase would hasten the rate of absorption of DDT without an equal increase in the rate of detoxification a lowering of resistance would occur. An increased rate of absorption of DDT might occur as a result of the SL larvae feeding faster than the CS or LL larvae, or as a consequence of greater physical activity of the SL larvae producing an increased exposure to DDT. Another possible reason for loss of resistance in the SL strain could be a decrease in the ability of the larvae to store DDT in fat bodies or in other non-sensitive tissues.

SUMMARY.—Selection of non-resistant *Drosophila melanogaster* for a short larval period line (SL), and a long larval period line (LL), resulted in a difference in larval period of some 18 to 20 hours between the two lines. Larvae of these selected strains and an unselected control strain were assayed for DDT-resistance at five separate generations of selection.

The slopes of the dosage-mortality regression lines varied from assay to assay but showed a consistent pattern with the SL strain steepest, the LL strain intermediate, and the control strain the shallowest. This relationship did not hold in generation 30 where the controls had the steepest slope. The differences among strains in the slopes of the dosage-mortality regression lines were not significant in all cases. The LD_{50} value for the SL strain was less than that of the control or LL strains in all generations tested.

When the data of the separate generations were pooled and treated as a single test, the dosage-mortality regression line of the SL strain was significantly steeper than those of the LL or control strains. Based on the pooled data, the SL strain showed a significantly lower LD_{50} value than the LL or control strains. No significant difference in the slope of the dosage-mortality regression lines or in LD_{50} values was found between the LL and control strains.

Selection for longer larval period produced no significant change in DDT-resistance of the LL larvae over that of the control strain; however, selection for short larval period resulted in increased susceptibility to DDT of SL larvae as compared with the other two strains.

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The Effects of Insecticidal Poisoning on the Level of Cytochrome Oxidase in the American Cockroach[1]

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In a previous communication (Morrison & Brown 1954) it has been reported that DDT and many other insecticides show *in vitro* effects on the cytochrome oxidase of the American cockroach. Out of 26 compounds tested, 22 (including DDT) were inhibitory at 10^{-3}M, and 4 (but not DDT) were inhibitory at 10^{-5}M concentration. Similar effects on cytochrome oxidase *in vitro* have been reported for DDT and seven related compounds with house fly material (Anderson *et al.* 1954). Sacktor (1949) had originally found that the cytochrome oxidase level of normal house flies exposed to residues of DDT or methoxychlor was reduced by one-half in 2 hours, but returned to the normal level 5 hours after the exposure.

The present experiments were undertaken to ascertain whether DDT and certain other insecticides showed *in vivo* effects on the cytochrome oxidase of the American cockroach. Corn-oil solutions of DDT, methoxychlor, parathion, malathion and butoxy thiocyanodiethyl ether were injected into the haemolymph, and the coxal muscles were assessed for cytochrome oxidase content at regular intervals subsequently.

MATERIAL AND METHODS.—Adult males of the American cockroach, *Periplaneta americana* (L), were taken 2 to 4 months after the final moult; their body weight varied between 650 and 800 mg. The insecticides, purified by recrystallization or redistillation, were administered as solutions in corn oil (Mazola, Canada Starch Co.); 30 cu. mm. were injected between two posterior abdominal sternites with a No. 26 hypodermic needle. DDT and methoxychlor were administered at a dosage of 1.0 mg. per insect, parathion and malathion at 0.25 mg. per insect, and β-butoxy-β-thiocyanodiethyl ether (the active principle of *Lethane 384*) at 0.6 mg. per insect. At these dosages death occurred from 16 to 26 hours after injection. The experimental insects were kept individually in bottles without food or water for periods ranging up to 48 hours before being sacrificed under CO_2 anaesthesia.

At various times after injection of the insecticide, individual cockroaches were assessed for the level of cytochrome oxidase in their meso- and metathoracic coxal muscles by the method of Schneider & Potter (1943). Approximately 30 mg. of muscle (moisture content 82%) was homogenized in 1 cc. of glass-redistilled water and then diluted with sufficient 0.05M phosphate buffer to give a final concentration of 6.0 mg. of muscle per cc. An aliquot of 1.0 cc. of homogenate was incubated at 27° C. and pH 7.2 with 1.0 cc. of cytochrome c (2.4×10^{-4}), 0.3 cc. of sodium ascorbate (0.114M), and 0.3 cc. of aluminum chloride (4×10^{-3}M). The oxygen consumption was measured by the Warburg constant-volume method, the CO_2 having been removed by 20% KOH on filter-paper wicks. Successive 10-minute readings showed regular increments between 10 minutes and 40 minutes after temperature equilibration. From the average of these three increments, results were expressed as oxygen consumption per 10 minutes per mg. dry weight of muscle. The control figures for untreated adult male roaches ranged between 19.3 and 28.7; they showed good uniformity within individual experiments, the higher values being recorded at later dates.

In each experiment three to five cockroaches were employed, one of which was left uninjected as a control. This latter was congruent with a corn-oil-injected control since it had been previously established (from four samples) that injection of 30 cu. mm. of corn oil had not effected any change in the cytochrome oxidase activity 24 hours later (22.4 ± 0.5 versus 21.9 ± 0.6). The remaining 2 to 4 insects were injected with the insecticide at different times, so that they could be sacrificed for cytochrome oxidase assay at approximately the same time. For exact synchronization of digests, some samples were held as homogenates for a short time in an ice bath; it was found that no loss of cytochrome oxidase activity occurred in 45 minutes under these conditions.

The possibility that holding the insects without food and water during the observation period would decrease the cytochrome oxidase activity was submitted to test. The assessment values in cu. mm./10 min./mg. obtained with two insects at each point of time were as follows: 0 hrs., 22.9 ± 0.5; 24 hrs., 22.7 ± 1.0; 48 hrs., 24.5 ± 0.2. Therefore starvation for 2 days does not decrease the cytochrome oxidase activity.

It was frequently observed during preliminary experiments that large differences existed in the cytochrome oxidase levels of randomly selected male cockroaches. Since the lower oxidase values were observed to be associated with a lighter colour of the muscle and a lighter tanning of

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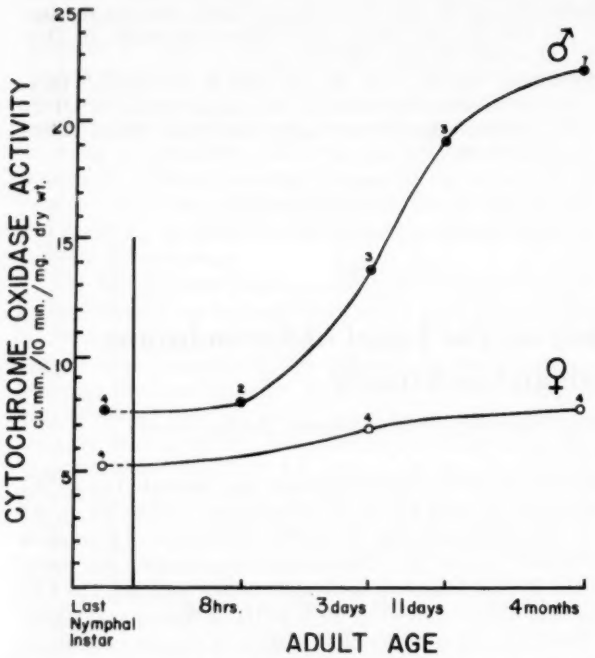

FIG. 1.—Relation of cytochrome oxidase activity with the age of adult *Periplaneta americana*.

the cuticle, the effect of difference in age on the level of cytochrome oxidase was determined. A total of 19 males and 12 females, distributed between various age groups, were assessed individually. The average values for each age group are plotted in fig. 1, the number of individuals being noted against each point, and the time scale being logarithmic. It is evident that the cytochrome oxidase activity of males rises to double the nymphal level within 3 days after the final moult, and at 11 days has almost reached the triple level that characterizes the old adults. In contrast, the cytochrome oxidase activity of females does not exceed the nymphal level by more than one-third, and does not increase appreciably after the 4th day of adult life. Furthermore, it was noted that the coxal muscle of male cockroaches showed a progressive darkening with age to a deeper pink and finally a red-brown colour; in female cockroaches, however, the change in muscle colour was comparatively insignificant. Similarly, the colour of the coxal muscles in females was considerably paler than that in males, corresponding to the difference in the cytochrome oxidase levels of the two sexes. As a result of these observations, all roaches employed in insecticide experiments were selected 2 to 4 months after the final moult, and most of the variation was thus eliminated.

RESULTS.—The effect of DDT on the cytochrome oxidase level of poisoned cockroaches is shown for the first 8 hours after injection in table 1. It will be seen that the insects passed through the typical stages of DDT poisoning without any significant change occurring in the level of cytochrome oxidase. The figures for cockroaches poisoned for longer periods are shown in table 2, the experiments having been performed at a later date. Here the levels for poisoned insects are significantly lower than the controls, the decrease in cytochrome oxidase level at 12 hours being 13% ($p < .05$). At 24 hours, when usually the insect was already dead, the decrease was 17% ($p < .01$), and at 48 hours after injection the decrease reached 30%.

Table 1.—Cytochrome oxidase assessment of male *Periplaneta*, in cu. mm./10 min./mg., 2 to 8 hours after injection of 1 mg. of DDT.

		Hours After Injection		
	Control	2	4	8
Stage of Poisoning:	—	excited, ataxic	tremors, falling on back	supine, spasms, twitches
Exp. 1	19.5	22.4	—	18.7
Exp. 2	24.2	21.8	22.9	24.7
Exp. 3	24.7	—	21.5	27.5
Exp. 4	21.5	23.3	24.5	—
Mean ± S.E.	22.5 ± 1.0	22.5 ± 0.4	23.0 ± 0.7	23.6 ± 2.1

The amounts of DDT that were present in the coxal muscle 4, 12 and 24 hours after injection were determined by the Schechter-Haller method. For the calibration curve, 30 mg. of coxal muscle had been added to the known standard quantities of DDT. Using this curve, the micrograms of DDT found in 30 mg. of coxal muscle at intervals after injection were 8 to 9 after 4 hours, 24 to 25 after 12 hours, and 12 to 13 after 24 hours.

The possibility that dilution of the DDT-poisoned muscle tissue during the assay process removed the inhibition by dissociating inhibitor from enzyme was tested in the following manner. A fairly concentrated homogenate was prepared from 150 mg. of coxal muscle homogenized in 1 cc. of distilled water and diluted with 4 cc. of phosphate buffer, and added to 5 cc. of cytochrome c solution and 1 cc. of $AlCl_3$ solution. After aliquots were removed as controls, this solution was incubated with DDT at 10^{-3}M concentration (prepared as an aqueous suspension as per Morrison & Brown) with shaking in a water-bath in 27° C. for 2 hours. Then aliquots were taken and diluted 1×, 2×, 4× and 10× with phosphate buffer and incubated with sodium ascorbate in Warburg flasks. The cytochrome oxidase assessments in cu. mm./10 min./mg. dry weight proved to be as follows:

Control	1×	2×	4×	10×
14.5	6.0	4.5	2.1	0.8

Thus the dilution of a DDT-poisoned tissue suspension in which the cytochrome oxidase had been inhibited by 59% did not result in any restoration of enzyme activity by removal of inhibition, but the reverse occurred.

The effect of methoxychlor on the cytochrome oxidase level of poisoned roaches is shown in table 3. The decrease is so slight that even 24 hours after injection, when usually

Table 2.—Cytochrome oxidase assessment of male *Periplaneta*, in cu. mm./10 min./mg., 12 to 48 hours after injection of 1 mg. of DDT.

		Hours After Injection		
	Control	12	24	48
Stage of Poisoning:	—	partially paralyzed	paralyzed or dead	dead
Exp. 1	27.5	23.8	22.2	21.1
Exp. 2	26.7	24.9	21.3	15.5
Exp. 3	25.8	19.1	22.0	18.7
Exp. 4	24.7	22.9	—	—
Mean ± S.E.	26.2 ± 0.5	22.7 ± 1.1	21.8 ± 0.2	18.4 ± 1.3

Table 3.—Cytochrome oxidase assessments of male *Periplaneta*, in cu. mm./10 min./mg., after injection of methoxychlor, parathion, malathion or butoxy thiocyanodiethyl ether.

		Hours After Injection			
	Control	4	12	24	48
Methoxychlor, 1 mg.					
Stage of poisoning:	—	supine, tremors, twitches	partially paralyzed	paralyzed or dead	dead
Exp. 1	28.2	—	28.7	24.4	16.6
Exp. 2	26.0	—	20.7	22.4	22.7
Exp. 3	22.9	22.0	25.8	24.0	—
Exp. 4	22.4	—	19.1	17.1	19.8
Exp. 5	24.7	24.7	20.9	19.5	—
Mean ± S.E.	24.8 ± 0.9	23.4 ± 1.0	23.0 ± 1.6	21.5 ± 1.2	19.7 ± 1.4
Parathion, 0.25 mg.					
Stage of poisoning:	—	supine, spasms, twitches	partially paralyzed	paralyzed almost dead	dead
Exp. 1	26.0	27.3	25.5	24.9	23.1
Exp. 2	27.1	28.9	25.5	26.9	22.2
Mean ± S.E.	26.6 ± 0.4	28.1 ± 0.6	25.5 ± 0.0	25.9 ± 0.7	22.7 ± 0.3
Malathion, 0.25 mg.					
Stage of poisoning:	—	supine, violent twitches	partially paralyzed	paralyzed almost dead	dead
Exp. 1	27.3	26.4	22.5	22.4	6.4
Exp. 2	26.9	27.1	28.4	22.0	4.7
Mean ± S.E.	27.1 ± 0.1	26.8 ± 0.2	25.5 ± 2.1	22.2 ± 0.1	5.6 ± 0.6
B-butoxy-B-thiocyano-diethyl ether, 0.6 mg.					
Stage of poisoning:	—	activity depressed	almost complete paralysis	dead	dead
Exp. 1	25.3	25.1	21.3	19.3	4.0
Exp. 2	25.8	27.5	26.2	18.7	4.9
Mean ± S.E.	25.6 ± 0.2	26.3 ± 0.8	23.8 ± 1.8	19.0 ± 0.2	4.5 ± 0.3

the insect was dead, it amounts to only 13% and is without statistical significance ($p < .10$). At 48 hours the decrease is 21% and significant at the 5% level.

The effects of parathion and malathion on the level of this respiratory enzyme are also shown in table 3. Parathion induced no reduction of cytochrome oxidase in the first 24 hours, when the insects proceeded through all the stages of poisoning almost to death; the reduction at 48 hours was only 15% ($p < .01$). On the other hand, malathion induced an 18% reduction at 24 hours ($p < .01$) and at 48 hours the reduction was 79%, accompanied by darkening and apparent lysis of the muscle tissue.

The effect of β-butoxy-β-thiocyano-diethyl ether (the active principle of *Lethane 384*) during the first 12 hours was to progressively suppress activity until paralysis was almost complete; yet it did not depress the cytochrome oxidase activity during this period (table 3). At 24 hours after injection, the insect being already dead, the enzyme activity had decreased by 26%; the decrease reached 82% at 48 hours, by which time the coxal muscle was very dark and had apparently disintegrated.

The observed decreases in cytochrome oxidase associated with insecticidal poisoning for the first 24 hours were compared with the decreases obtained 24 hours after the insect had been killed with an electric shock. Using three dry cells and an induction coil, a continuous high-voltage shock was applied with platinum electrodes to the head, and to the regions of the nerve cord and heart, which destroyed all response to stimuli and stopped the heart beat. The cytochrome oxidase levels of three cockroaches 24 hours after this treatment were 21.5, 20.2, and 19.8, being 19% lower than those of three control insects which were 26.0, 25.3, and 24.4 cu. mm./10 min./mg. When this decrease is compared with that of poisoned insects (table 4), it is seen that the only insecticide to induce a significantly greater decrease is butoxy thiocyanodiethyl ether ($t_3 = 3.6$, significant at the 5% level).

Discussion.—The preliminary work showed that there was an increase in the cytochrome oxidase content of the coxal muscle of male *Periplaneta* following the imaginal moult; the oxidase level had increased by nearly 200% at the end of the second week of adult life. Sacktor (1950) had observed that the cytochrome oxidase activity of *Musca* increased by 55% during the first 2 hours after emergence, and 118% during the first day. Although similar increases have been reported for other holometabolous insects (Bodenstein & Sacktor 1952, Ludwig 1953), no account of this phenomenon in a hemimetabolous insect could be found. Our experiments also show that the enzyme activity of the male *Periplaneta* is twice that of the female at 3 to 4 days and three times greater at 3 to 4 months after the final moult. Sacktor & Bodenstein (1952) had reported that male muscle of American cockroaches (of unstated age) showed 1.8 times the cytochrome oxidase activity of female muscle. These authors noted that this difference was associated with the male muscle being a darker pink in colour than the female muscle. In our experiments the increase in cytochrome oxidase in males has been shown to be associated with an increase in the red coloration of the muscle, while Ludwig *et al.* (1955)

Table 4.—Percentage decrease in cytochrome oxidase level 24 hours after treatment with insecticides or a lethal electric shock.

	CONTROL	EXPERIMENTAL	PER CENT DECREASE	P FROM CONTROL
Electric Shock	25.2±0.3	20.5±0.3	19	.01
DDT	26.2±0.5	21.8±0.2	17	.01
Methoxychlor	24.8±0.9	21.5±1.2	13	.05
Parathion	26.6±0.4	25.9±0.7	3	.10
Malathion	27.1±0.1	22.2±0.1	18	.01
Butoxy thiocyanodiethyl ether	25.6±0.2	19.0±0.2	26	.01

have reported that female muscle high in cytochrome oxidase activity was a deeper pink in colour.

The figures summarized in table 4 show that neither methoxychlor nor parathion induced a significant decrease in cytochrome oxidase *in vivo* in 24 hours, while the decrease caused by DDT and malathion was not significantly different from that produced by electric shock. The greatest decrease was associated with butoxy thiocyanodiethyl ether, which reached 82% at 48 hours after injection, and was accompanied by tissue necrosis. This thiocyanate insecticide immediately depresses the *in vivo* respiration of *Blattella* (Harvey & Brown 1951), and completely inhibits *in vitro* the cytochrome oxidase of *Periplaneta* (Morrison & Brown 1954). Malathion also showed an 80% decrease at 48 hours, accompanied by muscle necrosis. This organic phosphate completely inhibits cytochrome oxidase *in vitro* at 10^{-3}M (loc. cit. 1954), and has been found by O'Brien (in Spencer 1955) to inhibit the succinoxidase system in house flies.

The results show that DDT does not cause a significant decrease in muscle cytochrome oxidase activity while the insect passes through the typical stages of DDT poisoning. It cannot be argued that the DDT failed to reach the coxal muscles that were assessed, since the concentration of this insecticide reached almost 0.1% (25 micrograms per 30 mg. of muscle) 12 hours after injection. Nor is there any evidence that dilution of the tissue during assessment would mask an inhibition by dissociating it; the experimental figures show the reserve. The only conclusion is that DDT fails to inhibit *in vivo* the cytochrome oxidase activity of *Periplaneta*.

The observed inhibition of cytochrome oxidase by DDT *in vitro* remains to be explained. This has been reported for *Periplaneta* by Morrison & Brown (1954), for resistant and susceptible *Musca* by Anderson *et al.* (1954), and for *Tenebrio* and *Periplaneta* by Ludwig *et al.* (1955). The inhibitions *in vitro* reported by Sacktor refer to pork heart muscle (Sacktor 1949), and his subsequent reports (vide Chadwick 1952, on p. 406 and Perry & Sacktor 1955, on p. 330) do not state that insect tissue was used as the source of cytochrome oxidase. It is a characteristic of the *in vitro* inhibition of insect cytochrome oxidase that it is greatest when the tissue concentration is lowest. Ludwig *et al.* (1955) found that whereas DDT at 10^{-3}M inhibited 1% homogenates of *Tenebrio* by 50% it did not inhibit 10% homogenates at all. In the present investigation, incubation of 10^{-3}M DDT with the equivalent of a 1% homogenate of *Periplaneta* induced 59% inhibition in 2 hours; while its incubation with 0.2% homogenates (Morrison & Brown 1954) achieved 100% inhibition in 100 minutes.

It is a second characteristic of the *in vitro* effect of DDT on cytochrome oxidase that a certain period of exposure is required in order that the inhibition may accumulate. Morrison & Brown (1954) found that inhibition commenced after 50 minutes and became complete at 100 minutes; in a recent repetition of this experiment at this laboratory the inhibition was found to commence immediately and become complete 100 minutes later. Ludwig *et al.* (1955), also working with *Periplaneta* muscle and 10^{-3}M DDT, but without reduced cytochrome c, obtained 30% inhibition immediately, 50% after 1 hour, and =72% after 2 hours. Sacktor exposed a soluble cytochrome oxidase preparation (species source unstated) to DDT in the cold for 1 hour and obtained a 50% inhibition (Perry & Sacktor, 1955).

A third characteristic of the *in vitro* effect of DDT is that it requires concentration of the order of 10^{-3}M. The fact that concentration of 10^{-5}M and below are ineffective indicates that a general rather than a specific inhibition is involved.

These characteristics indicate that the inhibition of cytochrome oxidase by DDT *in vitro* is due to a physical process of adsorption of the hydrophobic DDT colloid particles on to the hydrophilic tissue enzyme, as suggested by Anderson *et al.* (1954). The DDT suspension, prepared by dissolving in acetone-ethanol and diluting in an aqueous solution of Triton X-100 emulsifier, is milky in appearance and sediments quite rapidly; the DDT therefore exists in the form of fairly large colloid particles. These would be able to inhibit dilute solutions of tissue cytochrome oxidase by adsorption, much as the hydrophobic colloid gum mastic is known to adsorb on to hydrophilic albumin colloid particles. When the homogenate concentrations are high, the hydrophilic tissue colloids could be adsorbed around the hydrophobic DDT particles, much as partially-hydrolysed albumin forms a protective colloid for a colloidal silver sol, and by thus removing it from the sphere of action could prevent the inhibition of cytochrome oxidase.

This hypothesis would serve to explain the non-specificity of inhibition of cytochrome oxidase *in vitro* found in several investigations. Of the 26 insecticides tested by Morrison & Brown (1954), no less than 22 were inhibitory at 10^{-3}M and all these formed colloid suspensions as prepared in water with Triton X-100 emulsifier. Of 11 bis(p-chlorophenyl) compounds tested by Anderson *et al.* (1954) *in vitro*, eight of them inhibited the cytochrome oxidase of *Musca* more than DDT, although most of them (including DDE) were not insecticidal at all. Cytochrome oxidase preparations (not stated to be of insect origin) have been inhibited *in vitro* by o,p'-DDT and o,o'-DDT as well as by DDE (vide Chadwick 1952). A test performed (with Morrison) in this laboratory had shown that even the nonchlorinated compound diphenylmethane was immediately and completely inhibitory when added as a suspension at 10^{-3}M to *Periplaneta* homogenates; we later saw the report of Anderson *et al.* (1954) that diphenylmethane inhibited the cytochrome oxidase of *Musca* homogenates.

We therefore conclude that DDT is without action on cytochrome oxidase *in vivo*, and that its *in vitro* effects are an artifact due to adsorption. We are therefore not surprised to learn that enzyme preparations from resistant house flies are no less susceptible to DDT inhibition *in*

vitro than those from susceptible house flies (Anderson *et al.* 1954) and that there is no correlation between cytochrome oxidase content and DDT-resistance when various strains of house flies are compared *in vivo* (Perry & Sacktor 1955). With regard to the recent finding that 10^{-5}M DDT *in vitro* partially inhibits oxidative phosphorylation and oxidation of Krebs cycle compounds in *Musca* (Sacklin *et al.* 1955), we would note that the DDT-ethanol mixture used forms an opalescent colloid system in water which could adsorb on to the unprotected subcell particles these authors employed.

SUMMARY.—Adult male *Periplaneta americana* were injected with lethal doses of DDT, methoxychlor, parathion, malathion and butoxy thiocyanodiethyl ether in corn oil. The cytochrome oxidase activity in the coxal muscles of poisoned insects decreased slightly in 24 hours, but only with butoxy thiocyanodiethyl ether was the decrease significantly greater than with insects killed electrically. Malathion and butoxy thiocyanodiethyl ether, but not the other insecticides, showed marked post-mortem reductions 48 hours after injection. It is concluded that DDT does not affect cytochrome oxidase activity *in vivo*, and that *in vitro* inhibitions are due to non-specific adsorption. Cytochrome oxidase increases markedly in males, but not in females, after the final moult.

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Fate of P32-Labeled Malathion Sprayed on Jersey Heifer Calves[1]

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Interest in the direct application of organophosphorus insecticides of relatively low mammalian toxicity, such as malathion, *O,O*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorodithioate, to warm-blooded animals has been stimulated by very favorable results from the direct application of malathion dusts or sprays to poultry for the control of ectoparasites (Vincent *et al.* 1954, Furman *et al.* 1955). Recent studies on the extent of contamination of meat and eggs from hens fed or sprayed with malathion formulations have shown that residues of malathion are of a low order and that malathion is rapidly metabolized and eliminated, principally as water-soluble ionic metabolites and degradation products (March *et al.* 1956). The experiments described below were undertaken in order to assess the extent of contamination in various tissues of an animal, the Jersey heifer calf, following the direct application of malathion as a water-emulsion spray.

MATERIALS AND METHODS.—*Tracer Preparation.*—The synthesis of P32-labeled malathion from neutron-irradiated red phosphorus was accomplished according to the method previously described by March *et al.* (1956). The initial relative activity of the labeled malathion was 8.03 c.p.s./γ, permitting the detection of less than 0.01γ of P32-labeled malathion at the beginning of the sampling period and of less than 0.02γ at the end of the sampling period. Examination of the labeled malathion by paper chromatography showed that the radioactive portion was 93 to 94% malathion.

[1] Paper No. 906, University of California Citrus Experiment Station, Riverside, California. Part II of a series of papers on the fate of malathion in warm-blooded animals and insects. This work was supported in part by grants from the American Cyanamid Company, New York, N.Y., and the U. S. Atomic Energy Commission, Contract AT(11-1)34, Project 6. Accepted for publication March 6, 1956.

Experimental Animals.—Two 3-months-old Jersey heifer calves weighing about 200 pounds each were used in these experiments. The calves were housed in a small shed with an adjoining corral and were fed alfalfa hay and a calf starter (Shur-Calf; General Mills, Minneapolis, Minn.). An emulsion concentrate was prepared containing 57% P^{32}-malathion, 32% xylene, and 11% emulsifier (Triton X-100). A water emulsion (0.5 per cent by weight) was prepared by adding 8.32 gm. of this concentrate to 938 ml. of water. Each heifer was sprayed with approximately 1 pint of the water emulsion applied by means of a 3-gallon pump-up-type compressed-air sprayer equipped with a fine-spray nozzle, at an initial pressure of about 60 p.s.i. The calves were completely covered with the spray, 1 pint per animal being sufficient to cause some runoff. Two applications were made 2 weeks apart, and the calves were sacrificed 1 and 2 weeks after the second application. Each calf therefore received a total of approximately 4.74 g. of P^{32}-labeled malathion in two applications 2 weeks apart. At an estimated weight of 200 pounds per calf, this was a total application of about 50 p.p.m.

Tissues.—The animals were killed, skinned, and halved. One half of each animal was divided into 10 standard veal cuts for radioassay; the other half was divided into three cuts for chemical assay (see fig. 1 for diagram of cuts used). In addition, samples of tongue, brain, spinal cord, thymus, thyroid, pancreas, kidney, liver, heart, rumen, suet, bone marrow, and hide were obtained for radioassay. The major cuts were prepared for assay by removing the meat, including any fat, from the bones and processing these tissues through a fine meat grinder. The larger organs noted above were processed in this same way; the smaller organs were subsampled by cutting small portions from a number of different areas.

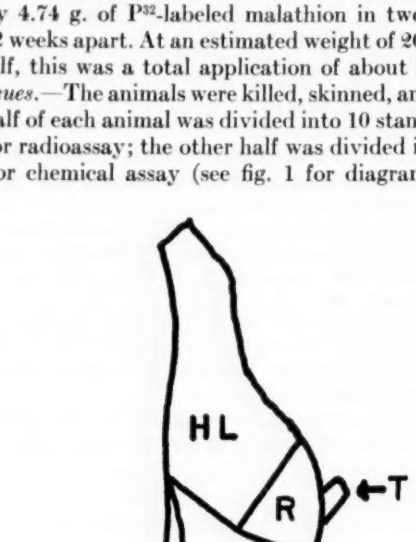

FIG. 1.—Standard veal cuts into which heifers were divided: FL, foreleg; SRC, shoulder rib chops; RC, rib chops; LC, loin chops; B, breast; TR, trimmings; R, rump; HL, hindleg; T, tail; and tenderloin (not shown).

Radioassay.—One-gm. subsamples of the various tissues were homogenized in 3 ml. of water in an all-glass Potter-Elvejhem homogenizer, shaken with 3 ml. of chloroform, and centrifuged for 10 minutes at 25,000 times gravity to separate the two phases and the insoluble residue; 1- to 2-ml. aliquots of the two phases were then taken for radioassay. The aliquots were placed in stainless-steel cupped counting planchets, 1 inch in diameter and $\frac{3}{16}$-inch deep, and were evaporated under a gentle stream of air and counted by means of a standard Geiger-Müller thin end-window tube. Quantitative values after correction for dilution, background, and progressive decay, were obtained by comparison with radioactive malathion standards.

A second set of 1-gm. samples was weighed directly into the cupped counting planchets, wet-ashed with concentrated HNO_3, muffled at 450°–500° C., and counted for total radioactive residue. All samples were prepared and counted in duplicate.

For comparative purposes a third set of samples was evaluated by digesting 5-gm. subsamples of certain tissues in a mixture of 3 ml. of concentrated HNO_3 and 10 ml. of water. Total activities in the resulting solutions were determined by means of a Geiger-Müller liquid dip counter (D52; Nuclear Instrument and Chemical Corporation, Chicago, Illinois), and quantitative values were obtained by comparison with a standard curve derived from fortified tissue solutions.

Chemical Assay.—Chemical analyses of malathion residues in the cuts of meat were accomplished by the carbon disulfide modification of the colorimetric method of Norris *et al.* (1954) suitable for the determination of malathion in lean beef and poultry meat. This method has been satisfactorily used to determine added amounts of malathion in lean beef in the range of 0.5 to 2.0 p.p.m. (American Cyanamid Co. 1955).

Urine.—At various intervals after the second spray application urine samples were collected in a beaker during elimination. No attempt was made to collect the urine quantitatively. Feces were not examined, studies on mice having shown that elimination of malathion from topical or injected doses is almost entirely through the urine. Urine samples were evaluated for total activity by wet-ashing 1 ml. of urine with concentrated HNO_3, and for chloroform-and water-soluble components by shaking 3 ml. of urine with 3 ml. of chloroform and examining aliquots of the two phases in a manner similar to that noted above for the tissues.

Other Techniques.—Techniques such as those used in paper-chromatographic separation of malathion and its metabolites, radioassay, and partitioning between immiscible solvents have been described elsewhere (Metcalf & March 1952, Metcalf *et al.* 1954, March *et al.* 1956).

Experimental Results.—The P^{32}-malathion and metab-

Table 1.—P^{32}-malathion and metabolites excreted in urine of heifer calves at various intervals after two spray applications each (two weeks apart) of 1 pint 0.5 per cent P^{32}-malathion water emulsion.

Hours After Second Spray	Residue in Urine (γ/Ml.)					
	Total P^{32}-Malathion and Metabolites		P^{32}-Malathion and Chloroform-Soluble Metabolites		Water-Soluble Metabolites	
	Heifer No. 1	Heifer No. 2	Heifer No. 1	Heifer No. 2	Heifer No. 1	Heifer No. 2
21	25.3	21.0	0.082	0.054	24.1	21.4
48	9.84	2.32	.22	.045	9.80	2.42
117	0.96	1.22	.010	.007	0.94	1.23
140	1.73	2.08	.014	.014	1.65	2.02
164		1.57		.038		1.50
213		0.73		.011		0.68
237		0.62		.023		0.56

olites excreted in the urine of the two heifers at intervals after the second spray application are shown in table 1. The greatest amount of activity appeared in the urine within the first 24-hour period, after which the amount of activity gradually decreased. The partitioning data in table 1 show that 96 to 99% of the malathion eliminated was in the form of water-soluble metabolites and degradation products. Previous studies (March *et al.* 1956) have shown that unchanged malathion partitions more than 70 to 1 in favor of chloroform from water, and that nearly all the metabolites and degradation products remain in the water fraction.

The data for the first 24 hours are particularly significant because they indicate that the greatest absorption of the applied malathion occurred within that period and that the absorbed malathion was rapidly metabolized, degraded, and eliminated. Although no data were obtained on elimination after the first spray application, the small amount of activity found in the urine of Heifer No. 2 ten days after the second application suggests that most of the malathion applied in the first spray had been eliminated before the second spray was applied.

The data in table 1 indicate that malathion is more rapidly and completely absorbed, degraded, and eliminated in the heifer than in the laying hen (March *et al.* 1956). Paper-chromatographic studies of the metabolites and degradation products in the heifer urine showed the same general patterns as those previously found in chicken droppings and mouse urine (March *et al.* 1956).

The distribution of total P^{32}-malathion and metabolites in the tissues of the two heifers 1 and 2 weeks after the second spray application is shown in table 2. There is excellent agreement in the data obtained by both the wet-ashing and the dip-counting techniques. All the tissues except bone were homogenized and partitioned between chloroform and water. No activity was found in the chloroform fraction of any of the tissues except the hide. (0.15γ/g. in the chloroform fraction and 9.12γ/g. in the water.) This shows that only water-soluble metabolites and degradation products were present in detectable quantities in the various tissues, except the hide, 1 and 2 weeks after application. The small amount (2.7%) of labeled compounds partitioning in favor of chloroform for the whole unwashed hide, including hair, is very surprising and indicates that after 2 weeks only negligible quantities of unchanged malathion remain on the exterior of the animal.

Table 2.—Total P^{32}-malathion and metabolites in tissues of heifer calves given two spray applications each (two weeks apart) of 1 pint 0.5 per cent P^{32}-malathion water emulsion.[a]

Tissue	Total P^{32}-Malathion and Metabolites in Tissues[b] (γ/g.)			
	Heifer No. 1		Heifer No. 2	
	Wet-ashed	Dip-counted	Wet-ashed	Dip-counted
Foreleg	0.07	0.06	0.12	0.11
Shoulder rib chops	.06	.07	.13	.06
Rib chops	.05	.05	.11	.08
Loin chops	.05	.05	.13	.08
Breast	.07	.05	.12	.10
Trimmings	.06	.07	.12	.10
Rump	.06	.06	.13	.08
Hindleg	.07	.06	.14	.11
Tenderloin	.07	.06	.15	.05
Tail	.07	—	.14	—
Tongue	.17	—	.18	.15
Brain	.08	—	.12	—
Spinal cord	.09	—	.13	—
Thymus	.44	—	.57	.62
Thyroid	.20	—	—	—
Pancreas	—	—	.46	—
Kidney	.18	.16	.24	.26
Liver	1.20	.99	.97	1.24
Heart	.13	—	.19	.39
Rumen (tripe)	.42	—	.34	—
Suet	—	.06	—	.10
Bone	—	.77	1.37	1.91
Marrow	—	—	.058	—
Hide	—	—	3.24–18.50	—

[a] Heifer No. 1 sacrificed 1 week after second spray; heifer No. 2, 2 weeks after second spray.

[b] Experiments in which homogenized samples of tissues were partitioned between chloroform and water showed no detectable activity in the chloroform phase except in samples of hide, in which a maximum of 2.7% of the total activity partitioned into the chloroform.

The residues found in the various cuts of meat and in the brain and spinal cord show a very uniform distribution of metabolites and degradation products throughout the animal. The somewhat higher values for the tongue are probably due to the heifers' habit of licking themselves. The levels of activity in the glandular thymus, thyroid, and pancreas indicate that a portion of the malathion must be degraded sufficiently to be utilized in the phosphorus metabolism of these organs, which are rich in phosphorylated compounds. The activity of the liver in metabolizing organophosphorus compounds is well known. The heifer liver is no exception for it contained the highest activity found in any of the soft tissues. Bone ranked next to hide in amount of activity, and here again is seen the utilization of phosphorus from the degraded compounds in normal metabolic activities of the animal.

The total activities found in tissues of heifer No. 2 were higher than those in tissues of heifer No. 1, even though heifer No. 2 was sacrificed a week later than No. 1. This difference may have been due to a number of factors but it can most likely be attributed to biological variation in the animals themselves. For example, heifer No. 1 excreted considerably larger amounts of activity in the urine in the second 24 hours after the spray application than heifer No. 2 did. It does not seem reasonable to conclude that the difference in activities in the tissues of the two

Table 3.—Chemical analyses for malathion in tissues of heifer calves given two spray applications each (two weeks apart) of 1 pint 0.5 per cent P^{32}-malathion water emulsion.[a]

Sample		Volume of Extraction Solvent (Ml.)	Per Cent Transmittance[b]	Malathion Found (γ)
Tissue	Weight (G.)			
		Control		
Fortified solvent blank (324γ. malathion)	—	200	43.9	324
Fortified rump (324γ. malathion)	200	400	48.9	275[c]
Fortified foreleg (162γ. malathion)	200	400	73.5	138[c]
Nonfortified rump (control)	200	400	98.2	—
Nonfortified foreleg (control)	200	400	98.0	—
		Heifer No. 1		
Foreleg	174	348	99.8	—
	227	454	99.1	—
Hindleg	178	356	98.0	—
	203	406	98.1	—
Rump	224	448	98.0	—
	168	336	98.9	—
		Heifer No. 2		
Foreleg	225	450	96.3	—
	207	414	97.2	—
Hindleg	165	330	97.5	—
	203	406	97.1	—
Rump	212	424	96.5	—
	211	422	98.2	—

[a] Heifer No. 1 sacrificed 1 week after second spray; heifer No. 2, 2 weeks after second spray.

[b] Since all readings for nonfortified control and treated tissue samples are above 96% transmittance it is possible to say only that treated samples contain less than 0.2 p.p.m. of malathion.

[c] Recovery, 85%.

heifers indicate a progressive increase in accumulation of malathion metabolites and degradation products.

The data for both urine and tissue samples indicate that malathion is rapidly absorbed, metabolized, and degraded; that elimination is rapid at first and then gradually decreases; that certain amounts of phosphorus from the degraded compounds are utilized in the normal phosphorus metabolism of the animal; and that eventually the elimination rate is probably based upon the normal turnover of phosphorus in the body.

Results of chemical analyses for malathion in tissues of the foreleg, hind leg, and rump of the two heifers are shown in table 3. All the transmittance readings for both nonfortified control and treated tissue samples are above 96%. Such readings lack significance because of instrumental limitations. From the data, however, it is apparent that if as much as 0.2 p.p.m. of malathion had been present, the transmittance readings would have been below 96%.

SUMMARY AND CONCLUSIONS.—A study has been made of the fate of P^{32}-labeled malathion and metabolites in two Jersey heifer calves after two spray applications each (2 weeks apart) of 1 pint 0.5% P^{32}-malathion water emulsion.

The malathion was rapidly absorbed and metabolized and was eliminated, in the urine principally as water-soluble metabolites and degradation products (96 to 99%). The amount eliminated was greatest during the first 24 hours and then gradually decreased.

The two calves were sacrificed 1 and 2 weeks after the second spray application, and residues in 10 cuts of the meat and in tongue, brain, spinal cord, thymus, thyroid, pancreas, kidney, liver, heart, rumen, suet, bone, marrow, and hide were determined radiometrically. Residues in all tissues except the hide were in the form of water-soluble metabolites and degradation products, no unchanged malathion or chloroform-soluble metabolites being found. In the hide only 2.7% remained as unchanged malathion and chloroform-soluble metabolies 2 weeks after the second spray application. Total residues in the meat cuts were low ranging from 0.05 to 0.15 p.p.m. Higher residues (0.2 to 2 p.p.m.) were found in thymus, thyroid, pancreas, liver, and bone. The highest residues (3 to 18 p.p.m.) were found in the hide.

Chemical analyses for malathion in tissues of the foreleg, hindleg, and rump of the two heifers showed no detectable malathion (less than 0.2 p.p.m.).

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Inhibition of Development in the House Fly by 3,4-Methylenedioxyphenyl Compounds[1]

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The addition of piperonyl butoxide to house fly culture medium has recently been found to have a strong inhibitory action on the flies' development (Mitlin & Konecky 1955). This compound was equally effective against insecticide-resistant and -susceptible flies. Because it is one of a large group of compounds containing the 3,4-methylenedioxyphenyl group that have shown synergistic activity with pyrethrum, because most of these compounds are relatively nontoxic to warm-blooded animals, and many of them are inexpensive, several of them were selected to determine whether they too could inhibit the development of the house fly. In addition, it was sought to establish whether this group was a toxophore under certain conditions.

SCREENING TESTS.—The materials were chosen randomly, the sole requirement being that each contained the 3,4-methylenedioxyphenyl group. They either were commercial samples or had been synthesized by chemists of the Entomology Research Branch[3] in the course of other work.

Each material was incorporated in Chemical Specialties Manufacturers Association rearing medium (1953) by a method devised by Mitlin *et al.* (1954). Most of the compounds were screened for effectiveness against both susceptible and resistant flies at 0.25 and 0.125 per cent. However, some of them were not available in sufficient quantity to test on resistant flies. In all the tests the susceptible flies were of the 1948 National Association of Insecticide and Disinfectant Manufacturers strain, and the resistant flies were of the Orlando-Beltsville strain that had been selected for DDT resistance for about 160 generations. If development was completely inhibited, the concentration was reduced until a threshold value was found. The effects of the compounds on the larvae and pupae, and on the number of adults emerging and their reproductive ability were measured.

The results of these tests are given in table 1. For most of the compounds the results are given only for the lowest effective concentration. *n*-Propyl isome, piperonal, and sesamin showed no activity when tested against suceptible flies at 0.25%, and 3,4-methylenedioxyphenyl benzenesulfonate showed no effect at 0.125%. Eggs laid by the surviving adult flies were checked for viability by placing them on damp filter paper in closed petri dishes to see if they hatched. None of the compounds affected the viability.

The visible effect on the flies was similar to that reported for piperonyl butoxide (Mitlin & Konecky 1955).

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[2] The author gratefully acknowledges the aid of Anne M. Baroody in conducting the experiments herein reported.

[3] W. A. Barthel, Morton Beroza, and S. I. Gertler.

Table 1.—Compounds inhibiting development at indicated concentrations.

COMPOUND (R=3,4-METHYLENEDIOXYPHENYL)	SUSCEPTIBLE FLIES		RESISTANT FLIES	
	Pupation Delay	Emergence	Pupation Delay	Emergence
0.125 per cent				
1-R-1-Butanol	—	—	Complete inhibition	
4-R-3-Butene-2-one	1 day	61%	2.5 days	41%
N,N-Dibutylpiperonylamine	1 day	None	3 days	40%
Isosafrole	—	—	Complete inhibition	
3,4,-Methylenedioxybenzyl analog of allethrin	1 day	9%	2.5 days	41%
1-R-4-Phenyl-1-butanol	1 day	None	3 days	None
Piperonylic acid	1 day	—	—	—
Sesamol	3 days	—	—	—
Sulfone	—	—	Complete inhibition	
0.0625 per cent				
1-R-1-Butanol	Complete inhibition		Complete inhibition	
2-Butoxyethyl R acetal acetaldehyde	4 days	25%	—	—
Butyl R acetal acetaldehyde	—	45%	—	—
5-Butyl-5-ethyl-2-R-*m*-dioxane	Complete inhibition		—	—
Dihydrosafrole	Complete inhibition		—	—
Isosafrole	Complete inhibition		Complete inhibition	
2-Methoxyethyl R acetal acetaldehyde	—	54%	—	—
R ester ofsobutylcarbamic acid	—	45%	—	—
Piperonyl alcohol	—	31%	—	—
Sulfone	Complete inhibition		Complete inhibition	
0.0313 per cent				
Dihydrosafrole	—	—	2 days	52%
2-Ethylhexyl R ether	1 day	25%	—	—
Sesamol	—	—	2.5 days	—
Sulfoxide	2.5 days	None	3 days	—

Table 2.—Comparative effects of the 3,4-methylenedioxyphenyl compounds sulfoxide and sulfone on the development of the house fly.[a]

Compound (Per Cent by Weight)	Susceptible Flies			Resistant Flies		
	Mean Days			Mean Days		
	Larval Period	To Emergence	Per Cent Emergence	Larval Period	To Emergence	Per Cent Emergence
Sulfoxide						
0.0039	7	13	100	7	13	100
.0078	7	13	91	7	13	48
.0157	7.5	13.5	47	8	14.5	27
.0313	9	15	3	9.5	15	13
Control	7	14	—	7	13	—
Sulfone						
0.0039	7	13	97	9	13	83
.0078	8	14	56	7	13	77
.0157	8.5	14.5	26	7	13	52
.0313	8.5	14.5	2	7	15.5	8
Control	7	13	—	7	13	—

[a] Two replications.

The affected larvae were discolored and undersized. All of them reached the third instar before their development was inhibited.

Comparison of Sulfoxide and Sulfone.—Two of the most effective compounds, sulfoxide and sulfone, were chosen for further tests of comparative effectiveness, and run in graduated series against both strains of flies. Dosage-effect curves for these compounds were plotted. The data on which these curves are based are found in table 2. Although comparisons were not made on flies from the same populations, valid comparisons can be drawn from the data.

On susceptible flies sulfone appears to be more effective than sulfoxide, the median effective doses being 0.010 and 0.017%. On resistant flies the relationship is nearly the same, 0.010% for sulfone and 0.015% for sulfoxide.

Discussion.—Although investigators at the Boyce Thompson Institute (Harvill & Arthur 1943, Prill *et al.* 1946, 1947) have shown that certain compounds containing the 3,4-methylenedioxyphenyl group are toxic to flies, this group has been generally thought to be of value only in the formulation of synergists in house fly insecticides. From the data given above it appears that compounds containing this group are toxic in their own right when incorporated in C.S.M.A. medium.

The Boyce Thompson Institute investigators, using the Peet-Grady method, found that high concentrations of the chemicals were required to show toxic effects. By the method reported here extremely low concentrations were effective in inhibiting development. For instance, they found isosafrole to have a low order of toxicity when used as a fly spray (Harvill & Arthur 1943), whereas by the method employed here the same material was highly toxic, the median lethal concentration being 0.0235%. Of greater interest is the fact that resistant flies are as readily affected as susceptible flies, as noted earlier for piperonyl butoxide (Mitlin & Konecky (1955).

Although these tests do not prove that the 3,4-methylenedioxyphenyl group is toxophoric, they add to the evidence that this is so. For with but few exceptions, all these compounds, randomly chosen, were toxic. Nor does there appear to be any definite relationship between synergism and inhibition. Many of the inhibitors are good synergists (Gersdorff *et al.* 1945, 1948), but this is not generally true.

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Some Factors Affecting Pollination of White Dutch Clover[1]

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The expansion of grassland farming in Mississippi has greatly increased the acreage of leguminous forage plants. Since these plants depend largely upon insects for pollination, a greater demand has been placed on the insect pollinators present whether the crops are managed for commercial seed production or reseeding of the pastures. This study is a part of a program initiated to investigate the pollination of such leguminous forage plants.

White Dutch clover and crimson clover are the most important of the early season grassland legumes in Mississippi. White clover was selected for this study because it is more widely used, and the results of much of the work with it might be applicable to crimson clover. Also there is more information available in the literature concerning crimson clover. Much work to date has dealt with alfalfa and red clover in the more arid regions of the country. Less work has been done in the southeastern states where competition for pollinators by other species is a serious problem. A number of investigators have reported on tests where yields of seed from plants caged to exclude bees were compared with yields from uncaged plants. Other workers have reported on the yields from plants with bees caged on them as compared with yields from plants in exclusion cages or with open check plots. In most tests the results were expressed in pounds of seed produced per acre.

Vansell (1951) reported on pollination of Ladino clover (a selection of white clover) in California. Seed production was reported in his paper in terms of seeds produced per pod or matured floret. This study also mentioned the relatively small number of bees, expressed as bees per square yard, actually found working the clover which was attributed to the low nectar production of the plants.

Methods and Materials.—The tests conducted in cages in 1952, 1953, and 1954, are described by numbers as assigned below, and will be so referred to subsequently in tables and text. Each year there were five replications of each test.

1. Insects excluded from the clover by a cage 1 yard square in area and 18 inches tall, covered by 16-mesh screen.

2. Insects excluded by a cage 1 yard square in area and 18 inches tall, covered by 8-mesh hardware cloth through which honeybees could not enter.

3. A shade 6 feet square made of 16-mesh screen was mounted 18 inches above the ground to allow naturally occurring insects to visit the clover. The 1 square yard area under the shade was delimited by boards for the purpose of making counts.

4. Honeybees from an attached nucleus of three standard frames were made to work under a cage of 6×9 feet in area and 3 feet tall, made of 16-mesh screen, with an access door. One yard square was delimited by boards for counts as in No. 3.

5. A check, partially defruited, was included in 1953 and 1954. An attempt was made in this category to have the blooms per square yard, delimited by boards, equal to the number found in the comparable replication of number 4.

6. A check plot of 1 yard square, delimited by boards, was used each year.

In 1952, the test was located on a good stand of white clover established for 2 years. In 1953 and 1954, plants grown in the greenhouse in 1-quart cans were set out in February on well fertilized, fallowed land. The plants used were clones produced by cuttings from two plants selected for early blooming and distinctive leaf characteristics. Two plants of each parent type were planted on each square yard of the test plots. This was necessary due to the high degree of self-incompatability of white clover pollen. The yield of both plant types was mixed in harvesting. Since the heads were a random, homogeneous mixture, the results should not be affected by the methods used for evaluation. The earliest blooms were removed from the plants until blooming was fairly general, The cages were set up in April each year.

The honey bee nuclei with each cage of number 4 were checked periodically and brood, bees, and food were supplied from colonies located near the test. These nearby colonies insured a plentiful supply of honey bees for the exposed numbers 3, 5, and 6.

At intervals throughout the season the number of honey bees working on each test area and the number of blooms having white or open florets were recorded. Other tests indicated that when a floret was pollinated, it turned a dark color and turned down before the next day. From the number of bees and blooms per square yard, a ratio of honey bees per 100 blooms was calculated. These data are presented in table 1. No honey bees were found in the exclusion cages, but the only other significant variation from the check is number 4. In 1952 and 1953, the ratio of bees-per-100-blooms in number 4 was greater than the check, and in 1954, it was less. This may cause a lack of confidence in the technique at first mention, but it may be noted that the relative activity, as indicated by the ratio of bees-per-100-blooms, is nearly equal for number 4 each year. The data also indicated a considerable variation in the activity on the exposed areas. This is a point of special interest, since it appeared to be correlated with the clover crop variations in the locality. There was a good honey crop in 1952, a very light crop in

Table 1.—Average numbers of White Dutch clover heads harvested and honey bees-per-100-blooms for the years 1952–54.

Treatment	Honey Bees per 100 Blooms			Total Heads		
	1952	1953	1954	1952	1953	1954
1. 16-mesh exclusion	0	0	0	630.2	634.2	269.6
2. 8-mesh exclusion	0	0	0	591.6	680.6	260.0
3. Screen shade	1.09	2.47	3.64	960.2	937.6	348.0
4. Caged bees	2.38	3.66	2.79	564.6	655.8	383.8
5. Partially defruited check	—	2.74	3.86	—	1081.6	378.2
6. Check	1.19	2.16	4.60	952.0	1230.2	390.6
L.S.D. at 5% level	0.55	0.58	1.18	172.2	134.2	114.2
at 1% level	0.76	0.78	1.61	234.4	183.1	155.7

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1955, but in 1954, most beekeepers reported no honey surplus. Although the clover production in the test was best in 1953, it was not true of the area as a whole, due to lack of plant survival.

Note in table 1 that the exclusion cages (numbers 1 and 2) reduced the head production each year. Number 4 reduced production in 1952 and 1953. Also in 1953 number 3 reduced production and number 5 reduced production at the 5% level of significance. Production in 1954 was very low due to dry weather most of the growing season.

Caging generally reduced the number of heads produced, and after this fact was noted in 1952, the partially defruited area (number 5) was added as an additional check. An attempt was made to have the same number of blooms left on each replication as was found in the same replication of number 4. Usually the number of white blooms in the exclusion cages was greater than on other areas, although the total head production was less. This was due to the lack of pollination which caused the florets to remain open for extended periods. Unpollinated heads in the exclusion cages had very fine, weak stems when they were harvested.

All dry heads were picked about every 2 weeks, and each picking was kept separate. Each plot picking was sampled for 50 heads, and all heads were counted. The early and late pickings of some plots had less than 50 heads, therefore data from these samples were adjusted to 50 heads for statistical studies.

Since white clover may produce as many as six seeds per pod, a study of the variation in production with relation to position of the pod on the head was made. It was found that well-pollinated heads had significantly more seeds per pod on the lower florets which bloom and mature first. For this reason partially shattered heads were rejected and replaced by whole heads in sampling.

In 1952 and 1953, there were five pickings, but in 1954, there were only three pickings. In 1952 the weight of the seed in a sample and the weight of 1000 seeds from the same sample were used to estimate the total number of seeds; however, in 1953 and 1954 all seeds were counted for greater accuracy. All seeds were weighed in 1953 and 1954, so the weight per 1000 seeds could be more accurately determined. The number of pods in each sample was counted to determine the seeds per pod and to study their variations under different conditions.

RESULTS.—The production of white clover seeds on an area may be affected by the number of heads, the number of pods, and the number of seeds produced per pod. All of these variables may be affected by the condition of the plants. For this reason data concerning certain plant responses to some of these interrelated variables in these tests are presented.

Of the three variables mentioned, the number of seeds per pod is the only one directly affected by pollination. In table 2, the seasons' average number of seeds per pod may be compared for the 3 years and six categories. Each of these numbers is an average of widely ranged data much like that for the check in 1953 presented in table 5. These data indicate that as the season advanced less seeds per pod were produced. In the exclusion cages 1 and 2, a more or less reverse pattern of production occurred. This indicates that the decline noted in table 5 was largely due to seed maturation.

Referring again to the number of seeds per pod recorded in table 2, it is found that in 1953 the check produced more than the others. Also it should be noted that there was no difference in number 4 and the check in 1952 and 1954. One objective of this treatment was to get maximum production, and since there was more bee activity in the cage in 1953, it seems evident that conditions other than pollination must have been involved. This effect was encountered again when an attempt was made to devise a simpler method of determining the seeds produced per pod by sampling the pods to determine the percentage having no seeds.

Table 2.—Seasonal average number of White Dutch clover seeds per pod and pounds per acre.

Treatment	Seeds per Pod			Pounds per Acre		
	1952	1953	1954	1952	1953	1954
1. 16-mesh exclusion	0.08	0.01	0.01	5.58	1.44	1.28
2. 8-mesh exclusion	.38	.27	.82	60.96	35.50	58.54
3. Screen shade	1.67	1.78	2.22	458.62	480.90	217.72
4. Caged bees	1.61	1.57	2.24	237.46	280.56	248.88
5. Partially defruited check	—	1.77	2.24	—	606.94	241.92
6. Check	1.59	1.92	2.21	385.18	676.40	252.98
L.S.D. at 5% level	0.18	0.08	0.29	54.42	154.34	77.56
at 1% level	0.24	0.11	0.40	74.98	210.50	105.78

In 1953, all pod samples were sub-sampled, and the pods in the sub-samples were broken apart on a sandpaper board with a hardwood peg to determine the percentage of seedless pods. The F values for both linear and quadratic correlations were highly significant.[2] A formula for the line was derived, and in figure 1 a graph of the values found in the test is plotted. Each value used in the calculations was the average of the five replications.

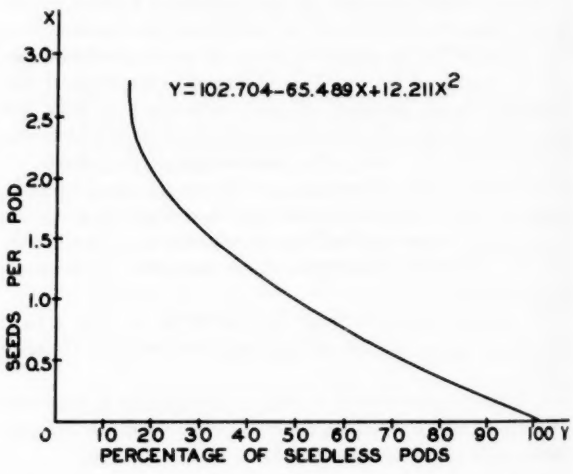

Fig. 1.—Relationship of seeds per pod and percentage of seedless pods in 1953.

Preliminary studies have shown that more than two seeds per pod were found in clover which was considered to yield a commercially valuable seed crop. A study of figure 1 shows that at about the two-seeds-per-pod point, less than 3% decrease in seedless pods resulted in about a 30% increase in seeds per pod. Due to the nature of the curve, it may be expected that about 10% of the pods

[2] The author wishes to acknowledge the assistance in fitting these data to the curve, by Dr. Walter J. Drapala, Statistician, Mississippi Agricultural Experiment Station.

Table 3.—Percentage of production of White Dutch clover heads and seed crop (by weight) on five picking dates, 1953.[a]

TREATMENTS	PICKING DATES				
	May 11	May 21	June 11	June 24	July 9
	Heads				
1. 16-mesh exclusion	0	15	39	28	18
2. 8-mesh exclusion	0	24	34	27	15
3. Screen shade	5	15	37	32	11
4. Caged bees	3	18	45	23	11
5. Partially defruited check	4	17	41	30	8
6. Check	7	20	45	25	3
	Seed (by weight)				
1. 16-mesh exclusion	0	2	5	53	40
2. 8-mesh exclusion	0	1	36	38	25
3. Screen shade	8	17	49	24	2
4. Caged bees	6	33	48	10	3
5. Partially defruited check	6	18	55	20	1
6. Check	11	23	47	18	1

[a] Data rounded to facilitate comparisons.

would not be pollinated, but extrapolation for more seeds per pod by the formula is not possible. Therefore, since the bees-per-100-blooms in the test was as great as is usually encountered, it would seem that the percentage of seedless pods cannot be used as a simple measure of pollination efficiency for field use.

To study the effects of the treatments on the production of heads and weight of seed, a percentage of the total of each was calculated for each picking in 1953. These data are presented in table 3. Examination of the data from the check shows that nearly half of the heads and seeds were produced at the third picking, which was made at about the average date of harvest. The usual rule-of-thumb harvest date is when three-fourths of the heads are dry.

In contrast to the check, which followed a normal distribution curve pattern, the exclusion cages 1 and 2, have a skewed distribution of both heads and seeds. This is of particular interest in view of the more or less normal distributions for number 4. This delayed fruiting for exclusion cages was therefore not due to the cage effect on the plant, but due to the lack of pollination. The seed production of the 8-mesh exclusion cage started earlier than in the 16-mesh, and the production was much heavier, as shown in table 2 where the seed weight is calculated on a per acre basis for the season.

Specimens of the families Halictidae and Andrenidae have been captured while working white clover, and it was noted that these small bees were sometimes seen in the 8-mesh exclusion cages, but never in the 16-mesh covered cages. Since the seed production in the 8-mesh cages increased as the season advanced, it seems likely that these insects were responsible for much of this seed production.

The total production was higher in these tests than it would be usually under field conditions, but comparisons under the various pollination conditions can be made. Too few seeds for good pasture reseeding were produced in 16-mesh exclusion cages, but with 8-mesh exclusion cages, apparently far more than enough were produced. Field counts in pastures indicated that about half of the head production was lost in grazing, and in our tests head production was reduced about the same by the caging. It therefore would seem unnecessary to recommend the use of additional honey bees for reseeding pastures when as many as 0.25 seeds per pod are produced, which would be over 30 pounds of seed per acre. The usual seeding rate is from 2 to 3 pounds per acre for starting new pastures.

Table 4.—Influence of treatments on seed weight and pod production in White Dutch clover in 1953.[a]

TREATMENT	PODS PER HEAD	GRAMS PER 1000 SEEDS
1. 16-mesh exclusion	68.0	0.2685
2. 8-mesh exclusion	62.8	.3882
3. Screen shade	65.6	.4299
4. Caged bees	65.0	.4312
5. Partially defruited check	70.6	.4118
6. Check	64.4	.4288
L.S.D. at 5% level	3.6	0.0457
at 1% level	4.9	0.0623

[a] Pods from third picking, June 11.

These tests do not give direct information about the number of colonies of honey bees needed for maximum seed production. It may be noted that highest seed yields were obtained in the check and only about 4.6 bees-per-100 blooms were found in this treatment, but this was not true for all years of the test.

During the early phases of this investigation, it was not known that the number of florets per head of white clover was dependent on the physiological condition of the plant. In table 4, it may be noted that there was a highly significant increase in pods per head in the partially defruited check 5, as compared with the untreated check 6. It may be noted that there were significantly less heads produced on the defruited check plot 5, as reported in table 1. This same effect was found again as the season advanced, as reported in table 3. There was a highly significant decrease in pods per head in the last two pickings as compared with the June 11 picking harvested in the peak of production. If heavy production caused this reduction, the question arises as to why the peak picking on June 11 had such a heavy production of pods. This was probably due to the fact that the florets were initiated before the seed development took place, and were not inhibited by seed maturation. In table 5, it was shown that the seeds-per-pod production for the June 11 picking was rather high (though less than earlier in the season.) The blooms for this production peak occurred in May when growing conditions were favorable.

Another variable factor which was affected by pollination as well as plant condition, was the weight of the seeds. The only treatment which significantly reduced the weight of the seeds, was the 16-mesh exclusion cage 1; however, there was a trend toward lighter seed from the

Table 5.—Influence of seasonal variations and seed maturation in White Dutch clover on production of pods, seeds per pod, and weight of seeds in 1953.[a]

PICKING DATE	PODS PER HEAD	SEEDS PER POD	GRAMS/1000 SEEDS
May 11	61.6	2.77	0.4832
May 21	62.2	2.15	.4509
June 11	67.0	2.37	.4177
June 24	57.2	1.63	.4349
July 9	32.2	0.73	.3570
L.S.D. at 5% level	6.0	0.27	0.0299
at 1% level	8.3	0.37	0.0412

[a] Data from check plots.

8-mesh exclusion cage also. See table 4 for details. Then in table 5, using the third picking on June 11 as a base, there was a decrease in seed weight from the second picking, which was significant at the 5% level. Therefore it would seem that under the strain of heavy seed maturation, the plant was unable to produce seeds as heavy as those maturing earlier in the season when the number being produced was less.

DISCUSSION.—When these tests were planned, no method could be devised to control various numbers of honey bees working the plots in order to get a gradient effect on pollination, and since the literature available seemed to indicate that 16-mesh screening had little effect on the environment in a cage made of this material, it was felt that the caged-bee treatment 4 would probably produce more seed than the check. The screen shade treatment 3 was added in the hope that it would allow fairly normal comparisons to be made between all the cages and the check, but it is evident from the data that caging caused environmental changes which were not simulated by a shade only. The humidity in the cages was increased, and this in turn affected loss of the soil moisture. Usually there seemed to be very little difference between the check and the screen shade treatment.

It seems that more honey bee activity caused more seed production when comparing the data in tables 1 and 2. There was more bee activity per bloom in 1954 than in 1953, and the seed production per pod was higher in 1954. On the other hand, table 2 shows that the pounds per acre of seed produced in 1954 was very low. Also in 1953 the average number of pods per head in the peak picking in the check was 64.4, and in 1954, there were only 56.0. The same parent plants were used. This would indicate that the physiological condition of the plants was different when floret initiation took place in the 2 years; therefore, a fair comparison of these data cannot be made.

The same hypothesis might be substantiated by the data presented in table 5, where the seeds per pod on the check decreased from the first to the third picking. Since the number of heads increased tremendously (table 3), the competition for pollination also increased, so it might be reasoned that the production decrease was due to less efficient pollination. This argument cannot be refuted on grounds of different numbers of pods produced the same year on the same plot, (table 4), but it should be pointed out that in the fourth and fifth pickings the head production was much less than in the third picking, yet the seeds-per-pod production was significantly lower in both of the later pickings. Therefore, the whole question again seems to be related to the physiological condition of the plants.

Some of the data presented in this paper indicate that since each of the white clover pods may produce at least 6 seeds, that if pollination occurs, it may be enough to produce one or more seeds per pod. As was shown in figure 1, when 80 to 90% of the pods in this test had any seeds, the production per pod would range along a steep, upward curve from about two seeds per pod. A scattergraph of the data without the use of the curve fitting formula, seemed to support the view that any insect pollination at all probably involved more than one pollen grain, since there was a sharp incline in the plottings near the 90% seedless-pods-point when the data came from the exclusion cages. The pollination for this very low production from that point down to 100% seedless pods might have been due to wind, water, or to causes other than insects, so that single pollen grains might have been transferred.

The study of pollination efficiency in the field is complicated by several natural factors. Tests were conducted in the greenhouse in which plants grown in flowerpots were used to prove that the seed production potential was affected by soil water, and various fertilizer elements; such as phosphorus, potassium, and boron. Also, field collections of head samples seemed to show a trend of variations in production from different soil types.

Pastures in Mississippi do not seem to need honey bees added for reseeding white clover. Examinations of random samples from different parts of the state in 1952 and 1953 showed that in no sample either year was the seeds per pod ratio less than 1.4. The highest ratio recorded was 3.17 seeds per pod.

The effect on seed production, of weather unsuitable for honey bee activity, was simulated in a small test in 1953. The technique employed was not very successful, but it was found that seed production per pod was reduced if pollination was not available to florets for several days. It is interesting to speculate what the effect on seed production would be, if several days of inclement weather occurred during the relatively short peak of bloom production. Unless the weather conditions seriously affected the growth of the plants, it seems likely that the plants would later compensate for much of the loss of seed by an extended fruiting cycle and by producing more seeds per pod in heads already initiated, and by also producing larger heads.

SUMMARY.—To study the effect of increased acreage of legume pasture on the pollination needs in Mississippi, cage tests were conducted in 1952, 1953, and 1954 to determine the effects of various levels of pollinators on white Dutch clover seed production. Treatments included: exclusion of pollinators by 16- and 8-mesh screen cages, 16- mesh sideless cage, 16-mesh cage with bees added, a check uncaged and a check which was partially defruited. Caging generally reduced head production. The 8-mesh exclusion cage produced more seeds than the 16-mesh, but both produced less than the other treatments. In 1953, the production of seeds per pod decreased as the season advanced. A correlation between the number of seeds per pod and percentage of seedless pods in a sample was found to be of no value in the field in predicting the production of seeds per pod from the more simply arrived at percentage of seedless pods. This was due to the nature of a curve fitted to the data in 1953.

Exclusion cages extended blooming, and the low seed production in these cages came late in the season. The 8-mesh exclusion cage allowed production of enough seed per acre to reseed pastures, but the 16-mesh exclusion cage did not. The production of pods per head varied with the different pickings. The partially defruited area produced more pods per head than the untreated check.

Seed weight varied through the season and was reduced by the 16-mesh exclusion cage.

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Lygus Bug Injury and Control on Carrot Seed in Northern California[1]

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One of the earliest references to seed injury by lygus bugs in the United States is that of Essig (1926), who states: "According to R. H. Smith the tarnished plant bug often injured seed of alfalfa, causing it to shrivel and dry up in the hull in Idaho, where it also attacks strawberries and raspberries." The actual importance of these insects in limiting alfalfa seed production has been shown since then by the intensive investigations of Sorenson (1932, 1936, 1939), Shull *et al.* (1934), Carlson (1940), and Stitt (1940). Hills (1941) showed the importance of these insects in sugar beet seed production, and was one of the first to report (1944) that 10% DDT dust was very effective in controlling adults and nymphs of *Lygus oblineatus* (Say). Michelbacher *et al.* (1944) also reported on the control of lygus bugs with DDT; within 2 years many workers had investigated the use of DDT for control of this insect, as shown by the review of R. F. Smith & Michelbacher (1946).

This type of work has continued at an ever increasing pace on a number of seed crops. Flemion *et al.* (1949) reported that *L. oblineatus* (Say) caused embryoless seeds in dill plants and probably in carrots. They first suspected this in 1947, and after cage tests with several insects in 1948, they proved that this bug was at least one of the *Lygus* spp. responsible for embryoless seed. Handford (1949) also reported that *L. campestris* (L.) was a major factor in reducing carrot seed yields in British Columbia, and found that the application of DDT materially increased yields. Further investigations by Flemion & Olson (1950) definitely pinned the embryoless seed condition on lygus bugs for several umbelliferous seed crops; they also stated that these bugs can greatly reduce seed yields. The two early reports by Flemion *et al.* cited here also contain an excellent review of the literature dealing with the many types of damage caused by lygus bugs to a number of crops. Further work by Flemion & MacNear (1951) gave additional data on the adverse effect on plant growth and reduction of seed yield caused by the feeding of these bugs. A number of other entomologists also have noted that lygus bugs have caused varying degrees of seed loss and decreases in germination in various vegetable seed crops.

Horticulturists and seedsmen in the Sacramento Valley of Northern California, where considerable carrot and other vegetable seed is grown, found that one or more applications of DDT dust reduced their seed and germination losses to some extent. These measures were stimulated by reports of the work cited above on alfalfa and the umbelliferous seeds in other parts of the country. However, the exact effect, the extent of seed loss, the number of lygus bugs necessary for an economic loss, and the timing and number of insecticidal applications were not known for this area.

The present report describes the more important results of investigations during 1954 and 1955 on the relationship of *Lygus hesperus* Knight[2] to Red Core Chantenay carrot seed grown in this area. This insect is the principal economic species. *L. elisus* Van Duzee is also present, but to date has been of minor importance on the carrot seed crop.

Kelton (1955) places the lygus bugs of economic importance in the genus *Liocoris* Fieb., 1858. However for the present it seems proper to retain the use of the genus *Lygus* Hahn, 1883, because of the petition: "A proposal to use the plenary powers to fix the type species of the genus *Lygus* Hahn, 1883," by J. C. M. Carvalho, H. H. Knight, and R. L. Usinger, to the International Commission on Zoological Nomenclature.[3]

The number of generations and length of the life cycle of *Lygus hesperus* on carrot seed was determined and plotted from nymphal and adult bug counts made in the check plots of the field control investigations. The type and extent of carrot seed damage by these bugs was investigated by means of replicated cheesecloth field cages enclosing varying numbers of seed heads. Different numbers of bugs were tested per seed head to determine the population density necessary to effect economic seed losses. Nymphs were also tested in these small cages to determine any differences in the amount of damage caused by the adults versus the nymphs. Field control tests during the first season's investigations were timing tests comparing treatment with 5% DDT dust at 25% of bloom and at 25% of petal fall. The second season's control investigations consisted of replicated single row plots separated by buffer rows. These tests compared one, two, and three applications of 10% DDT dust; and compared 10% DDT and 10% toxaphene at two applications.

SEASONAL CYCLE ON CARROT SEED.—*L. hesperus* nymphs and adults were studied in both the 1954 and 1955 seasons to determine the number of generations in a single season and the length of the life cycle on carrot seed at Davis, California. The results are shown in figure 1. They indicate that there were two distinct generations in 1954, the first nymphal peak occurring on June 11 and the second on July 16. The adults developed slowly and were most numerous on July 9. The second generation took approximately 35 days for development. From this point on, the population declined rapidly because the carrot seed plants were fast reaching maturity.

The 1955 counts also showed that there were two generations for that season, but the results were not as clear-cut since the counts were made in the checks of the replicated field plots and were influenced somewhat by the dusts. The first nymphal peak seemed to occur on June 16, and the second was distinctly on July 13. The adult population was leveled off by the dusts and did not show a distinct peak. The nymphal counts indicated that

[1] Accepted for publication March 16, 1956.

[2] Determinations were made by Dr. Robert L. Usinger, Professor of Entomology, University of California, Berkeley. Field determinations were then necessarily made by the author for the cage and control investigations.

[3] Kelton (1955) states that if this petition is accepted the following will be effected: "*Lygus* Hahn, 1883, containing the type *Cimex pabulinus* L. and the species of *Neolygus* Knight, will become *Lygocoris* Reut., 1875. *Liocoris* Fieb., 1858, containing the "Lygus" bugs of economic importance, will become *Lygus* Hahn, 1883, with *pratensis* (L.) as type."

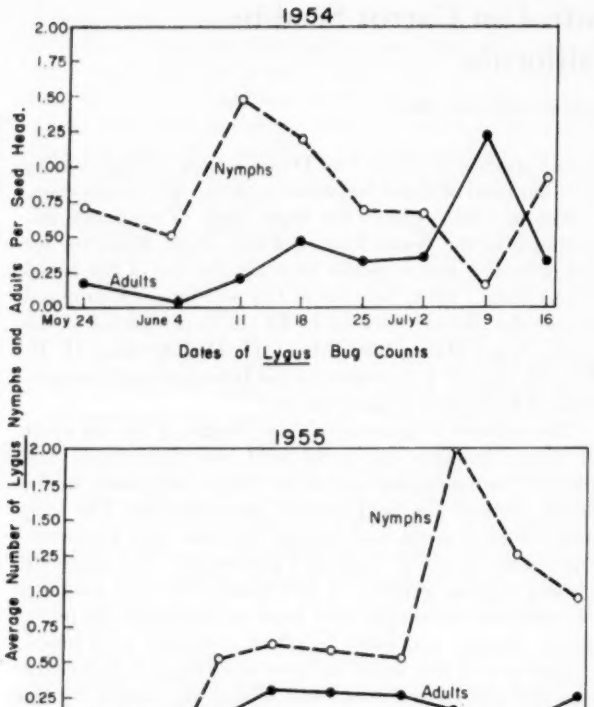

FIG. 1.—The seasonal cycle of *Lygus hesperus* Knight on carrot seed at Davis, California.

in 1955 the second generation developed in about 27 days.

Thus, the results indicate that the second generation of lygus bugs develops in 27 to 35 days on carrot seed in this area. This period of total development, from egg deposition to the adult stage (actually from egg to egg), corresponds very well with that found on alfalfa seed by other workers in warm, irrigated areas. Stitt (1940) found that the complete life cycle in Arizona at 85° F. took about 27 to 30 days (this included a pre-oviposition period of 7 to 10 days). Shull (1933) in Idaho reported the complete life cycle there, under slightly lower temperatures, as 35 to 38 days. Sorenson (1939) in Utah, carrying on studies at even lower mean temperatures and an elevation of 5,000 feet, found that development there was 46 days and more. Because the findings of the present study agree so closely with previous reports, no detailed life history work was necessary on carrot seed in this area.

Other Biological Observations.—These bugs bred rapidly and well on the carrot seed crop. Counts showed that 99 out of 100 eggs, figure 2, were laid in the pedicels rather than the peduncle, or main stalk. Furthermore, counts made on one particular day showed that there were 0.2 bugs present per seed head in the seed heads in full bloom, 0.56 per seed head in those past petal fall, 2.74 bugs per seed head in those in the green spine stage, 0.77 bugs per seed head in those in the brown spine stage. This clearly showed a preference for the seeds in the green spine stage, where the microclimate and food are apparently ideal for these bugs. At this stage the seed heads form al-

FIG. 2.—A photograph of two *Lygus hesperus* eggs, about 23 times natural size, laid in a carrot seed pedicel.

most perfect shade from the hot sun, and the ovules are soft and succulent.

TYPE AND EXTENT OF INJURY TO CARROT SEED. The mechanics and physiology of lygus bug feeding will not be discussed in detail here, but can be referred to in the recent review by Elmore (1955). Of most recent interest and significance, however, is the work of Flemion *et al.* (1952), who have estimated the amount of salivary secretion deposited by lygus bugs when feeding; that of Ledbetter & Flemion (1954) on a new high-voltage shock method for obtaining piercing-sucking mouthparts of bugs in host tissues; and that of Flemion & Ledbetter (1954) on the movement of the stylets of these bugs in feeding. In the last of these reports they state: "By observing the movements of the stylets during feeding it was found that considerably more mechanical destruction takes place than had been postulated. A great deal of damage occurs as the needlelike, extremely flexible, and rapidly-moving stylets pass through and between the cells in the process of rupturing and emptying many cells."

Field Cage, Experimental Methods.—The effect and extent of *Lygus hesperus* injury to carrot seed in northern California was investigated in 1954 and 1955 by enclosing one to four seed heads in small cheesecloth field cages. Each cage was tied to a stout stake. The seed heads, all of about the same age, were placed inside the cage and sprayed with a 0.01% pyrethrum solution about a week before the bugs were introduced to eliminate extraneous insects. Cotton was used around the main seed stalks on the bottom tie. Each of the six replications of all treatments for a particular series was on a separate plant. The first series of cage tests was initiated, by introducing different numbers of lygus bugs per treatment, when the seed heads in each replication began blooming. The second series was begun after petal fall, when the first series was terminated. In the third series of small field

cages the lygus bugs were introduced when the seed heads began blooming and kept in them until maturity of the seed heads.

In 1954, one, three, or nine adult female lygus bugs were added to each cage. The supply was checked every other day and kept replenished. The bugs were introduced into the first series of cages when the umbels began blooming, and each replication was terminated when petal fall was reached, in 15 to 17 days. Bugs were not introduced into the second series of cages until after petal fall, and this series of tests was terminated in 17 days when the brown spine stage was reached. All cages were sprayed with pyrethrum again as each replication was terminated. During 1954 only one seed head of the second order of umbels was used per cage, and approximately five blowflies were added and kept in both series of cages for pollination. The number of adult bugs and blowflies was noted on each check date; dead bugs were removed and live ones introduced by means of a tube aspirator as needed. Puparia were added as needed to keep the blowfly numbers at a sufficient level to effect pollination. At maturity the seed heads were threshed and cleaned by hand, and all undeveloped seed as well as the whole and sound seed was saved. After getting the total weights per seed head, 100 seed units were counted per replication. Germination tests were run on these seeds, and the number of seeds per gram and per ounce was calculated. One replication was lost in the tests run after petal fall because one plant rotted.

The cage technique was essentially the same in 1955, but fewer bugs per seed head were investigated, and the effect of nymphs versus adults was tested. Other changes were: (1) adult male bugs were used rather than females, since the presence of a few gravid females resulted in the late development of a few first and second instar nymphs in some 1954 tests; (2) the period of bug testing was extended to 39 days (from the beginning of bloom to the brown spine stage); (3) two to four seed heads were used per replication; and (4) only the apparently sound and whole seed was weighed and used for germination tests (after hand threshing and cleaning). Two replications were lost in the adult series of tests because of contamination in one replication of all treatments and because of gophers.

1954 Field Cage, Experimental Results.—The 1954 cage experiments showed that the bugs caused a blasting of carrot seeds during the blooming period and embryoless seed after petal fall (table 1). These two basic types of injury are proved by the very significant percentage of empty seeds found in the germination trials for those tests in which the bugs were allowed to feed during the blooming period only, while the percentage of empty seeds was not significant for the tests in which the bugs were allowed to feed after petal fall only. These results showed that the bugs fed mostly on the seed embryos after petal fall, thus causing embryoless seed.

In the tests conducted during the blooming period, one adult bug per seed head caused a significant seed loss, over 60%, nine bugs caused an almost total loss, while three bugs caused no increase in seed loss over that of one bug (this is due to experimental error). Germination was decreased significantly by one bug per seed head at this time because of blasting of the seeds; further decrease in germination was significant and in proportion to the increase in the number of bugs. Tests conducted after petal fall showed that lygus bug injury at this time resulted in only about half as much actual seed loss as the bugs' feeding at blooming time. However, these seed losses were not significant if the three-bug-per-seed-head treatment is included, since experimental error showed no seed loss for this treatment. Germination was significantly lowered by bug feeding after petal fall when feeding was on the embryo, but on the whole was not decreased as much as it was during the blooming period. Since this reduction in germination is due to embryoless seed, however, and these seeds cannot be eliminated in the cleaning operation, bug control is doubly important.

Table 1.—The effect and extent of lygus bug damage to carrot seed, shown by the 1954 cage experiments.[a]

No. *Lygus* per Seed Head	Average Weight per Seed Head (Grams)	Per Cent of Seed Loss	Average No. Seeds per Gram	Per Cent of: Germinating Seed	Per Cent of: Empty Seed
1. *Blooming Period* (adult female bugs)					
A. Check	1.138	—	429.3	89.56	10.44
B. 1	0.430*	62.23	758.6	67.51	32.49
C. 3	0.432*	62.14	1028.8*	37.97	62.03
D. 9	0.062**	94.71	1443.6**	4.68	95.32
L.S.D. 5%	0.586*	—	525.29*	—	—
1%	0.810**	—	726.43**	—	—
2. *Petal Fall to Maturity* (adult female bugs)					
A. Check	1.321	—	494.46	89.02	2.43
B. 1	0.909	31.18	536.58	64.20	8.80
C. 3	1.436	0.0	990.28	48.60	11.80
D. 9	0.748	43.37	615.54	56.20	15.20
L.S.D. 5%	n.s.	—	n.s.	—	—
1%	n.s.	—	n.s.	—	—

[a] There were six replications of all treatments except for series 2, where one plant died from rot; this left five replications since each replication of all treatments was on a separate plant.

1955 Field Cage, Experimental Results.—The results of the 1955 cage experiments are given in table 2. These data showed that one adult bug per seed head was enough to cause a significant and economic seed loss of up to 54.9% which was only a little less than that obtained in 1954. One adult bug per two seed heads was just short of causing a significant seed loss but indicated that this concentration of bugs approaches economic loss, while one bug per four seed heads was not nearly enough to cause appreciable seed loss. One bug per seed head caused a decrease in germination which was significant at the 5% level but was not as low as in 1954. This was because no less than two seed heads were used per replication and a better seed set resulted; furthermore, no blasted or undeveloped seed was used, so there were no significant differences in numbers of empty seeds.

The nymphal tests also showed a significant seed loss at one nymph per seed head but not at one nymph per two seed heads, although the latter concentration indicated a seed loss double that resulting from the tests with adults. The decrease in seed germination was very significant at both one nymph per seed head and one nymph per two seed heads, which indicates some error in the yield data. Other data on the weight of 100 seeds units and the number of seeds per ounce substantiate this. As a result, the data suggest that lygus bug nymphs cause about twice as much damage to carrot seed as the adults. Thus, the 1955 cage experiments show that control measures for

Table 2.—The number of lygus bug adults or nymphs per seed head resulting in economic damage to carrot seed, shown by the 1955 cage experiments.[a]

Treatment	Average Weight per Seed Head (Grams)	Per Cent of Seed Loss	Average Weight of 100 Seeds (Grams)	No. of Seeds per Ounce	Average Number of	
					Germinating Seeds/100	Empty Seeds
			1. *Adult Male Bugs*			
A. Check. Two seed heads	3.34	—	0.162	17,500	98.50	0.0
B. One adult bug/seed head	1.51**	54.92	0.070**	40,500	87.00*	1.25
C. One adult bug/2 seed heads	2.95	11.94	0.097**	29,226	92.75	0.75
L.S.D. 5%	1.02	—	0.034	—	8.05*	—
1%	1.55**	—	0.052**	—	n.s.	—
AA. Check. Four seed heads	2.72	—	0.162	17,500	98.50	0.25
D. One adult bug/4 seed heads	2.54	6.61	0.125	22,680	93.50	0.75
L.S.D. 5%	n.s.	—	n.s.	—	n.s.	—
1%	n.s.	—	n.s.	—	n.s.	—
			2. *Third through Fifth Instar Nymphs*			
A. Check	3.78	—	0.213	13,380	97.50	0.33
B. One nymph per seed head	2.11**	44.18	0.131****	21,775	71.00***	4.5
C. One nymph per two seed heads	2.90	23.26	0.163**	17,484	82.83**	2.5
L.S.D. 5%	1.03	—	0.005	—	10.18	—
1%	1.47**	—	0.008**	—	14.48**	—

[a] Two replications were lost in the adult tests, leaving four; all six replications were used in the results of the nymphal tests.

lygus bugs should begin when the adult and nymphal bug count reaches one per two seed heads. Seed blasting or failure to develop, which occurs during the blooming and petal fall periods, is shown in figure 3.

FIELD CONTROL EXPERIMENTS ON CARROT SEED.—*1954 Methods.*—The 1954 control experiments consisted of two hand-dusted non-replicated plots, each having a relatively large check block. A 5% DDT dust was applied to each plot at two different times. One plot was dusted at 25% of bloom of the second stage of bloom of the seed heads (second order of umbels); the other plot was dusted at 25% of petal fall of the second stage of bloom. These times were determined by actual seed head counts from 10 plants of each treatment. Subsamples of 10 seed heads in each of five locations were taken for the bug counts, yields, and germination tests. Bug counts were made by quickly knocking each seed head into a funnel fastened to a quart jar and then inverting the jar into a small box

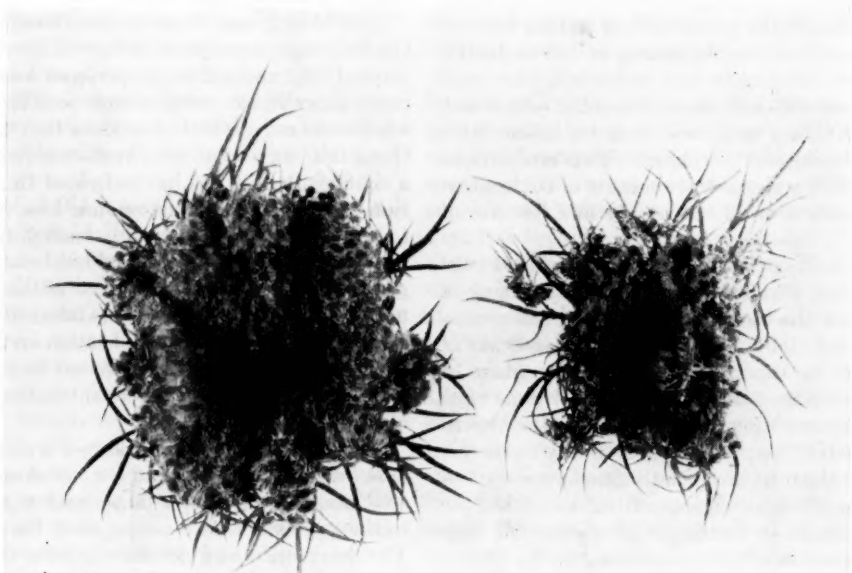

FIG. 3.—The blasting or lack of development of carrot seeds caused by lygus bug feeding; normal seed head on the left and damaged on the right.

Table 3.—The 1954 lygus bug control tests on carrot seed showing the relationship of timing with the stage of bloom of the seed heads.[a]

Treatment (Dust)	Average No. of Lygus per Seed Head and Control		Average Weight per Seed Head (Grams)		Average No. of Germinating Seeds per 100 Seeds	
	Seasonal Average	Per Cent Control	Second Order	Third Order	Second Order	Third Order
South Plot. Dusted 6/5/54; at 25% of bloom of second order of seed heads.						
5% DDT	0.50	62.40	5.95*	1.80	85.20**	57.60
Check	1.33	—	4.76	1.06	74.60	64.20
L.S.D. 5%	—	—	1.18*	n.s.	5.12	n.s.
1%	—	—	n.s.	n.s.	7.45**	n.s.
North Plot. Dusted 6/15/54; at 25% of petal fall of second order seed heads.						
5% DDT	0.55	43.87	5.05	1.74	80.40	66.40
Check	0.98	—	4.50	1.00	80.20	67.20
L.S.D. 5%	n.s.	—	n.s.	n.s.	n.s.	n.s.
1%	n.s.	—	n.s.	n.s.	n.s.	n.s.

[a] These were unreplicated tests, so five subsamples of 10 seed heads each were taken for all lygus bug counts and yield data. A seasonal count of *Orius* predators resulted in a mortality of 13.69% for the 5% DDT dust.

where the bugs and predators could be counted rapidly. Sweeping was not practicable because of the excessive seed head damage which resulted. The necessary seed heads for yields and germination were taken when the third stage of bloom of the seed heads was turning brown.

1955 Methods.—The 1955 control experiments consisted of single-row replicated field trials, with a buffer row between each replication. A 10% DDT dust was applied at 40 to 50 pounds per acre with a hand rotary duster. A comparison of different numbers of applications was made as follows: (1) one application, early in the blooming period of the second stage of bloom of the seed heads; (2) two applications, early in the blooming period of the second and third stages of seed heads; and (3) three applications, early in the blooming period of the second third, and fourth stages of seed heads. In addition, 10% toxaphene dust was compared with DDT at two applications early in the blooming period of the second and third stages of seed heads.

Lygus bug counts were made at eight different intervals after the first dust application for all treatments, by quickly shaking 25 seed heads from each replication into funnel fastened to a quart jar. Yield data for the 1955 tests were taken from 10 seed heads of each of the four replications, from the first, second, and third stages of seed heads and from 10 whole plants per replication cut at the brown spine age of the third stage. According to actual seed head counts made from 10 to 20 plants, the first dust application on June 6 was put on when the second stage of seed heads was 48.7% into bloom; the second application was made on June 17 when the third stage was 27.2% into bloom. The seed heads from the individual stages of bloom were threshed by hand while the whole plants were threshed by machine, and all were cleaned by means of a clipper seed cleaner.

1954 Field Control Results.—The results of the 1954 control tests are given in table 3. A significant (at the 5% level) increase in seed yield of the second stage of bloom was obtained in the plot dusted with 5% DDT at 25% of bloom of the second stage. There was no significant increase in seed yield when the carrot seed was dusted with 5% DDT at 25% of petal fall. The average lygus bug control for the season was 62.4%, which is only poor to fair overall control, but the germination was increased significantly (at the 1% level) to 85.2% for the second stage when the seed crop was dusted early in the blooming period. In the second plot only 43.9% seasonal control of the bugs was achieved, and there was no increase in germination when the plants were dusted after blooming. One application of 5% DDT dust brought about no increase in yields or germination for the third stage of seed heads, at either the early or late application period. Thus, it was evident that the first application of an insecticide to control lygus bugs on carrot seed must be made early in the blooming period of the second stage of seed heads, but that one application of 5% DDT dust is not enough for adequate control.

1955 Field Control Results.—The data obtained from the 1955 control investigations are given in tables 4 and 5. The average percentage of bug control was calculated from the seasonal average of the number of bugs per seed head. Treatment C, which involved three applications of 10% DDT, resulted in a good seasonal control, 93.3%. The control afforded throughout the season by one or two applications was only poor to fair, in direct relationship to the number of applications. One application resulted in poor control and agreed very well with the control ob-

Table 4.—Lygus bug control on carrot seed comparing one, two, and three applications of 10% DDT dust and DDT versus toxaphene dusts.[a] 1955.

Treatment	No. of Lygus Adults and Nymphs per Seed Head and Control		Average Weight of Seed per Seed Head for Three Stages of Bloom and for Whole Plant Yields (Grams)				
	Seasonal Average	Per Cent Control	First Order Umbels	Second Order Umbels	Third Order Umbels	Whole Plants	Pounds per Acre
A. 10% DDT, June 6	0.342	65.7	6.75	4.81	0.57	58.45	1677.6
B. 10% DDT, June 6 and 17	0.204	79.5	7.11*	4.98	0.77*	65.66*	1889.5
C. 10% DDT, June 6, 17 and 30	0.067	93.3	7.49**	5.71	0.96**	62.49	1798.3
D. 10% toxaphene, June 6 and 17	0.634	36.4	6.68	4.96	0.52	54.21	1560.0
E. Check	0.997	—	5.48	4.80	0.49	53.46	1538.4
L.S.D. 5%	—	—	1.32*	n.s.	0.21*	9.91*	—
1%	—	—	1.86**	n.s.	0.29**	n.s.	—

[a] Each treatment was replicated four times. Twenty-five seed heads per replication were used for each bug count, and 10 seed heads and 10 whole plants were used for yields and germination tests.

Table 5.—The effect of control experiments on the viability and number of carrot seeds.[a] 1955.

Treatment	Average Number of Germination Seeds per 100 Seed Units				Average weight of 100 Seed Units and the Number of Seeds per Ounce from Whole Plant Yields	
	First Order Umbels	Second Order Umbels	Third Order Umbels	Whole Plants	Weight of 100 Seed Units	Number of Seeds per Ounce
A. 10% DDT, June 6	96.0*	92.0	71.5	89.5	0.085	33,529
B. 10% DDT, June 6 and 17	96.0*	93.75*	74.0	95.0*	0.112	25,446
C. 10% DDT, June 6, 17 and 30	98.75**	98.5**	94.25**	97.5**	0.125**	22,800
D. 10% Toxaphene, June 6 and 17	89.25	84.0	63.5	89.5	0.085	33,529
E. Check	90.5	86.75	68.75	80.25	0.085	33,529
L.S.D. 5%	5.44*	5.44*	15.12	5.31*	0.028	—
1%	7.63**	7.62**	21.20**	7.45**	0.039**	—

[a] Germination tests were run on moistened blotting paper in plastic sandwich boxes, at 68° F. and 90% R.H. The most significant difference in number of seeds was in the third order, where, for example, treatment C resulted in 29,381 seeds per ounce while there were 50,000 seeds per ounce in the check. The differences in the percentage of empty seeds between the check and treatments were not significant.

tained in 1954 from one application put on at about the same stage of growth.

Three applications of 10% DDT, treatment C, resulted in a significant (at the 1% level) increase in yield for the first stage of seed heads, no significant increase in yield for the second stage, and again, a very significant increase in yield for the third stage. Because of experimental error this treatment did not result in a significant increase in yield for the whole plants, but it gave a highly significant increase in germination and produced the largest seed. Two applications of 10% DDT, treatment B, resulted in a lower significant (at the 5% level) increase in yields of the first and third stage of seed heads, no increase for the second stage, but an increase in yield for the whole plants (significant at the 5% level). Treatment B also resulted in a lower average increase in germination, with low and unsatisfactory germination of the third stage. Neither one application of 10% DDT nor two applications of 10% toxaphene resulted in any significant increases in yield or germination; these treatments were only a little superior to the check.

Figure 4 graphically shows the differences in bug control obtained in the 1955 control experiments. The marked increase in residual effectiveness of DDT over toxaphene is readily apparent. Toxaphene holds up satisfactorily for only about 8 days, while DDT is apparently capable of satisfactory control for as long as 23 days. Three applications of DDT are shown to be necessary for good bug control right up to the maturity of the carrot seed crop. Control with one application of DDT fell off too early for satisfactory results, but the DDT was a little better than the two application toxaphene treatment under the conditions of this experiment. Two applications of DDT dust were appreciably better and approached satisfactory control. When all the data are taken into consideration, the superiority of the three-application treatment of 10% DDT is shown. This experiment also indicates the advisability of using at least 10 replications to obtain reliable yield data, and of using larger blocks that would be less influenced by the dust treatments and migration of the lygus bugs.

Thus, two seasons' results from the control experiments have shown that it is necessary to apply an insecticide

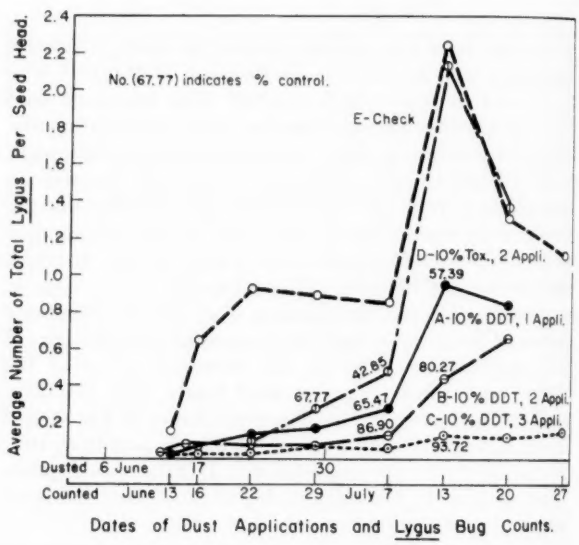

FIG. 4.—This comparison of four treatments shows the superiority of three applications of 10% DDT for lygus bug control on carrot seed, the residual effectiveness of DDT, and the inferiority of toxaphene for control at Davis.

early in the blooming period, at 25 to 50% in bloom, beginning with the second stage of bloom of the seed heads, to control lygus bugs in carrot seed at Davis, California. Three applications are advisable during early bloom of the second, third, and fourth stages of seed heads. The advisability of three applications becomes increasingly apparent when it is realized that the first three stages of seed heads ordinarily produce about 90% of the carrot seed crop. Ten per cent DDT dust is superior to 10% toxaphene in this area and will give satisfactory results if timed properly and if good coverage is obtained in the seed heads. The timing of the three 10% DDT dust control applications, correlated with the stages of bloom of the seed heads and number of bugs per seed head, during the 1955 season is shown in figure 5.

Effect of the Control Insecticides on Predators.—Additional data, given graphically in figure 6, showed that the

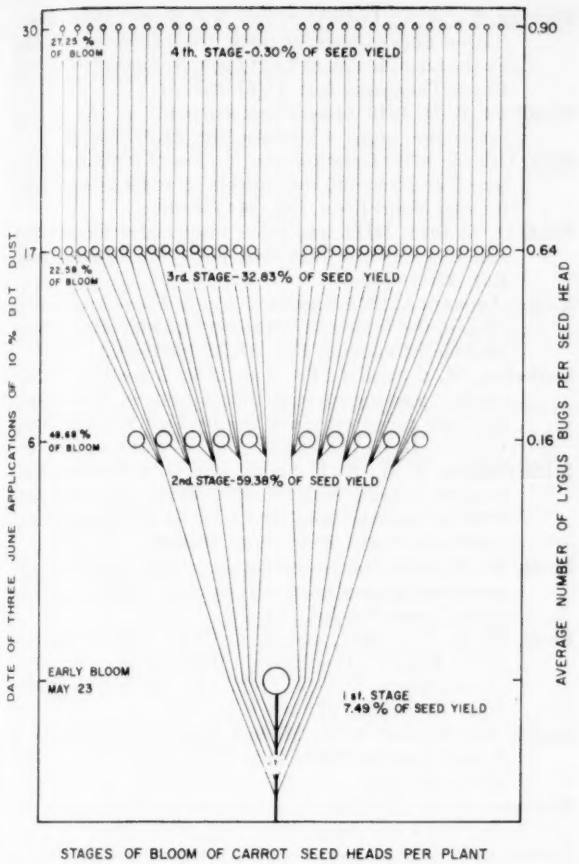
30
17
6
27.25 % OF BLOOM
4th. STAGE-0.30% OF SEED YIELD
22.50 % OF BLOOM
3rd. STAGE-32.83% OF SEED YIELD
48.68 % OF BLOOM
2nd. STAGE-59.38% OF SEED YIELD
EARLY BLOOM MAY 23
1st. STAGE 7.49 % OF SEED YIELD
0.90
0.64
0.16
DATE OF THREE JUNE APPLICATIONS OF 10 % DDT DUST
AVERAGE NUMBER OF LYGUS BUGS PER SEED HEAD
STAGES OF BLOOM OF CARROT SEED HEADS PER PLANT

FIG. 5.—A schematic drawing showing the timing of the three 10% DDT dust control applications, correlated with the stages of bloom of the seed heads and the number of bugs per seed head, during the 1955 season.

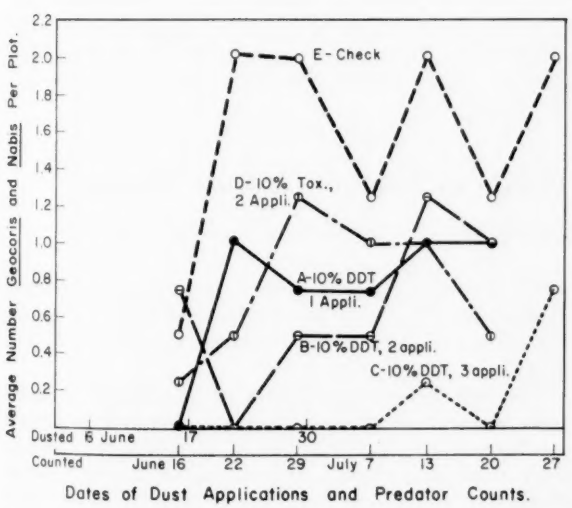

FIG. 6.—Compared with figure 4, this shows that with three applications of DDT the mortality of the lygus bug predators is about as great and lasts about as long as for the lygus bugs; but the predator population was too low for them to be a factor in lygus bug control.

predators *Nabis* sp. and *Geocoris* sp. were reduced the most by three applications of DDT. Their numbers decreased to about the same extent as the lygus bugs, and for about the same period. These predators increased fairly rapidly after only one or two applications, but their population density never became as high as the check. Toxaphene seemed to be a little less toxic to the predators, the seasonal mortality being the same with two applications as with DDT applied once. Actually, however, the predator population was low; under the conditions of this experiment predators were not an important factor in the control of the lygus bugs.

Data obtained in 1954 on the red spider predator, *Orius* sp., resulted in an average seasonal mortality of only 13.7% for one application of 5% DDT. Observations in 1955 indicated again that this predator is fairly tolerant to DDT, and no serious or even moderate red spider population developed on the carrot seed crop.

SUMMARY.—Two seasons of investigations with *Lygus hesperus* Knight on Red Core Chantenay carrot seed indicated that during one season there were two generations of this bug on the carrot seed crop in the Davis area. The second generation developed in 27 to 35 days, which corresponds closely to the time found by other workers in other warm irrigated areas.

The 1955 cage experiments showed that one adult bug per two seed heads is enough to cause minor economic carrot seed damage, and that nymphs cause about twice as much damage as adults. The 1954 results showed that these bugs cause a blasting or prevent the development of carrot seeds, especially during the blooming period, and that they primarily cause embryoless seed after petal fall. These investigations have shown that during the blooming period one adult or nymph per seed head caused losses amounting to 44 to 62%, and reduced germination to as low as 64%. The seed loss caused by bug feeding after petal fall was only about half as much, but germination can again be reduced to about 64%, and this is most serious since these embryoless seeds cannot be cleaned out. These findings show the need for insecticidal control measures when the bug count reaches one per two seed heads.

The 1954 control experiments showed that an insecticide should be applied early in the blooming period of the carrot seed crop. The first application should be made when the second stage of bloom of the seed heads is 25 to 50% into bloom, at which time an economically injurious bug population was ordinarily beginning to develop.

Control experiments comparing one, two, and three applications of 10% DDT dust were conducted in replicated field plots in 1955 and showed that three applications, early in the blooming period of the second, third, and fourth stages of seed heads, resulted in the most effective lygus bug control. The three-application treatment resulted in satisfactory bug control through maturity of the seed crop, with a significant increase in the seed yield for the first and third stages of seed heads, larger seed, and a consistently high and significant increase in germination. One application of 10% DDT dust did not provide satisfactory bug control, while two applications resulted in fair control with less significant increases in yield, size of seed, and germination than

three applications. Under the conditions of this experiment, two applications of 10% toxaphene were inferior to one of 10% DDT, were only one-third as residual, and were unsatisfactory for control.

The predators *Nabis* sp. and *Geocoris* sp. were reduced the most by three applications of DDT and seemed to be reduced to about the same extent and for the same period as the lygus bugs. Observations indicated that the red spider predator, *Orius* sp., is fairly tolerant to DDT.

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Studies on the Control of Boll Weevils in Surface Woods Trash[1]

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One of the most common hibernation sites of the boll weevil, *Anthonomous grandis* Boh., in the Southeastern States is trash found on the ground in the edge of woods. This trash consists of humus, decaying leaves, and pine needles. In the coastal section of South Carolina, boll weevils leave cotton fields from September through the first week in November and enter woods to hibernate. This movement begins before frosts occur and ends soon after the first killing frost.

Studies conducted at Florence, S. C., in 1938 (Bondy & Rainwater 1942) showed that 78% of the boll weevils hibernating in surface woods trash were found in the first 50 feet of the woods areas bordering cotton fields. Practically no weevils were found at distances greater than 150 feet from the edge. Further studies in 1953 (Fife & Walker, unpublished) showed that 84% of weevils hibernating in woods trash were within the first 100 feet of woods.

The satisfactory application of granulated formulations of insecticides from an airplane over difficult terrain has been reported (Farrar 1953). Members of the Plant Pest Control Branch's White-fringed Beetle Control Project of the U. S. Department of Agriculture have shown that an even distribution of particles can be obtained on the ground surface in wooded areas by aerial applications of granulated insecticides.

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[2] In cooperation with the South Carolina Agricultural Experiment Station.

Table 1.—Emergence of boll weevils in cages where surface woods trash was treated with granulated insecticides. Florence, S. C. 1955.

Insecticide (Per Cent)	Pounds of Active Ingredient per Acre	Number of Weevils Emerging[a]		Per Cent Emergence	Per Cent Net Mortality
		Total	Average per Cage		
Dieldrin 10	2.5	242	30.2	6.0	61.0
	5	136	17.0	3.4	77.9
BHC 5 (gamma)	5	32	4.0	0.8	94.8
	10	18	2.2	0.4	97.4
Untreated check		308	77.0	15.4	
L.S.D. at 5% level			5.1		
at 1% level			7.0		

[a] A total of 4,000 weevils caged for each treatment and 2,000 for the check.

These findings suggested the advisability of determining whether granulated insecticides would kill overwintering boll weevils in surface woods trash. Accordingly, two types of experiments were conducted—a large-scale field experiment at Holly Hill, S. C., in 1954 in cooperation with the White-fringed Beetle Control Project, and a cage experiment at Florence in 1954 and 1955. The results of these experiments are given in this paper.

LARGE SCALE FIELD EXPERIMENT.—The purpose of this cooperative experiment was twofold, (1) to determine the efficacy of aerial applications of several granulated insecticides in controlling the white-fringed beetle and (2) to study the effect of these applications upon the boll weevil in hiberation quarters and on seedling cotton.

A total of 548 acres infested with white-fringed beetles was treated with several granulated insecticides during the first week in March 1954. All crop land within this area was treated with heptachlor or aldrin at the rate of 2 pounds per acre. Ditches and railroad and highway right-of-ways within the infested area were treated with dieldrin at 4 pounds per acre. Wooded areas within and bordering the infested area were treated to a depth of 200 feet—14 acres with toxaphene, 30 acres with chlordane, 60 acres with dieldrin, 6 acres with aldrin, and 14 acres with heptachlor—all at the rate of 4 pounds per acre. All the granules had a particle size of 30/60 except those of dieldrin, which were 30/40. Applications were made with a Stearman airplane supplied by the U. S. Department of Agriculture Aircraft and Equipment Center at Oklahoma City, Okla.

The surface woods trash was examined in January and February to determine the number of live boll weevils present at the various locations where insecticides were to be applied. Post-treatment examinations were made in April at the same locations. A definite number of square yards of the trash was collected at each location and passed through a shaker machine to determine the number of live boll weevils present. Similar examinations were made in an untreated area.

Examinations for boll weevils were also made in all cotton fields within the treated area, each week beginning the first week in May. Records were also made in 12 fields of approximately the same size in an untreated check area about 4 miles from the treated area.

Pretreatment examinations in the areas to be treated with heptachlor, toxaphene, and chlordane disclosed live boll weevils present at rates of 968, 1694, and 2904 per acre, respectively. No boll weevils were found in the post-treatment examinations. In the area treated with

FIG. 1.—Applying a granulated insecticide to surface woods trash in cage in which hibernating boll weevils are confined.

dieldrin the pretreatment examination showed 564 live boll weevils per acre and the post-treatment count 387, a reduction of 31%. In the untreated check area the initial count was 2420 and the second count made at the same time as the post-treatment counts 1452 per acre, indicating a natural mortality of 40%. A woods fire burned out the area where aldrin was applied, making a post-treatment count impossible.

Counts on May 5, 11, and 20 showed 17, 15, and 21 boll weevils per acre, respectively, for the 12 fields in the treated area compared with 102, 83, and 26 for those in the untreated area. Infestations in some of the untreated fields were so great that the growers started their own control programs approximately 3 weeks before growers in the treated area.

CAGE EXPERIMENT.—In this experiment 36 pyramid-type screen cages (Fig. 1) were used, four of them as untreated checks. Each cage was 3 feet square at its base and had a removable top. All the cages were placed over woods trash at the edge of a wooded area. Those used for testing the insecticides were arranged in a randomized block in such a manner that there were eight replications for each four treatments, and the check cages were outside of the experimental area but adjacent to the test cages. Between October 18 and 28, 1954, boll weevils were collected in the field and 500 were placed in each cage.

On March 1, 1955, a large salt shaker was used to make a uniform application of an insecticide to the surface trash in each cage. The insecticides used were 10% dieldrin and BHC containing 5% of the gamma isomer. The dieldrin was applied at 2½ and 5 pounds and the BHC at 5 and 10 pounds of gamma isomer per acre. The granules of each insecticide had a particle size of 30/40.

The value of the treatments was determined by counting the living boll weevils that emerged between March 1 and June 24, examinations being made three times each week. In the cages treated with BHC emergence was practically complete by March 25, only four boll weevils emerging after that date. In the cages treated with dieldrin only three weevils emerged after April 20. In the check cages emergence continued through June 24. The difference between the two treatments with dieldrin was highly significant, but that between the BHC treatments was not significant. At both rates BHC gave much better control than dieldrin. Less than 1% of the boll weevils survived in the cages treated with BHC.

SUMMARY.—A large-scale field experiment and a cage experiment were conducted in South Carolina in 1954 and 1955 to determine the effectiveness of granulated insecticides applied to surface woods trash in controlling overwintering boll weevils. In the large-scale experiment, aerial applications made to surface woods trash in a small agricultural community reduced boll weevil populations considerably. The cage experiment demonstrated that a high kill of overwintering weevils could be obtained in this manner.

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The Biology of Three Chrysopid Predators of the Cotton Aphid[1]

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During the summer and fall of 1954, three species of lacewings, *Chrysopa oculata* Say, *Chrysopa rufilabris* Burmeister, and *Chrysopa plorabunda* Fitch, were observed in aphid-infested cotton fields near College Station, Texas. While these insects are well known for their predaceous habits and have been studied to some extent in various parts of the United States and Canada, relatively little is known concerning their activities in the cotton-growing areas of Texas. In view of this fact, studies were initiated in August, 1954 to determine the biology of the three species.

The life history studies reported herein were conducted in the laboratory at College Station and field observations were made in Brazos, Burleson and Robertson Counties. Detailed descriptions of all stages of these species of *Chrysopa* were presented by Smith (1922), therefore, it is not considered necessary to include further descriptions here.

MATERIALS AND METHODS.—Glass vials (25×95 mm.) were used to confine the various stages while obtaining records on the life history and habits. The cotton aphid, *Aphis gossypii* Glover, was used as food in most of the larval rearings and in all of the feeding tests. The turnip aphid, *Rhopalosiphum pseudobrassiace* (Davis), was abundant on cabbage and turnips throughout the winter and was used as a substitute food when cotton aphids were not available.

Regarding adults, *oculata*, the golden-eye lacewing, was the only one of the three species found to feed directly on aphids. Adult *rufilabris* and *plorabunda* were never observed to devour aphids but would readily feed on honeydew and various other liquids. Honeydew probably constitutes the major part of their food under natural conditions. Due to the difficulty encountered in obtaining and handling the necessary quantities of honeydew, the possibility of using an artificial food for these two species was investigated. Finney (1950) was able to increase egg production of *C. californica* Coquillett by feeding the adults on a mixture of protein hydrolysate of yeast (sold commercially as "MRT") and water. On the basis of this information, a suitable artificial food was developed utilizing materials available in the laboratory at that particular time. This mixture consisted of "DELMOR" nutrient

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powder, honey and water. It was mixed at the rate of 1 gram of powder to 10 cc. of equal parts of honey and water. Honey was added to give the mixture the needed consistency. The use of this protein-rich food may have increased egg production slightly above that to be expected when using honey dew.

Feeding tests were conducted in the laboratory to determine the number of cotton aphids consumed by larvae of the three species and also by adults of *oculata*. Known numbers of aphids were introduced into vials, each of which contained a single larva, and counts were made of aphids alive at the end of each 24-hour period. This procedure was repeated each day throughout the feeding period of the larvae. The same technique was used in conducting feeding tests with adult *oculata*.

SEASONAL ABUNDANCE AND OVERWINTERING.—*Chrysopa rufilabris*.—This species was the most abundant lacewing in aphid-infested cotton fields during August and September. Adults and larvae were collected on Johnson grass (*Sorghum halepense* (L.) Pers.) throughout October and November, although they never occurred on this plant in large numbers. A few adults were taken from alfalfa in December, 1954 and January, 1955, but no eggs, larvae or cocoons were found during this time. Several males and females were collected at lights in College Station February 1, 1955. A few of these females were confined to vials and each deposited a small number of eggs. Eggs and larvae were taken from oak trees during the first part of March.

Chrysopa plorabunda.—This species was never as abundant on cotton as *rufilabris*. Following the decrease of aphid populations on cotton, larvae and adults were collected on Johnson grass and weeds along fence-rows and roadsides. Several larvae were taken on cotton November 9 and on Johnson grass November 17, 1954. These were the last larvae of this species to be observed in the field until March, 1955. Adults occurred abundantly on alfalfa throughout the winter.

Numerous light brown adults of this typically green species were observed in alfalfa fields during December and at about the same time some of the adults under confinement in the laboratory also began changing color. In several instances, one or both members of a pair of adults confined to the same vial changed color. Brown-colored females were never observed to deposit eggs.

Chrysopa oculata.—The golden-eye lacewing was never as abundant on cotton as either *rufilabris* or *plorabunda*. It was found by laboratory tests that the larvae enter the soil to pupate. This explains why cocoons were never found on the plant as in the case of the other two species.

One adult was collected November 9 while sweeping weeds along a roadside and a few larvae were taken from Johnson grass November 17, 1954. These were the last members of this species to be collected from the field until March, 1955.

Twenty laboratory-reared third instar larvae were confined to an outside cage December 23. By the end of the month all had constructed cocoons about 3/4 inch below the surface of sand in the bottom of the cage. About one-half of the larvae died in the prepupal period and the others remained in this stage until the middle of February and pupated at that time. One adult emerged February 27. No other adults had emerged by March 20 and some appeared to have died in the pupal stage. In view of these facts, it is thought that this species overwinters in this area as prepupae in the soil.

LIFE HISTORY AND FEEDING HABITS.—*Chyrsopa rufilabris*.—The duration of the developmental stages is summarized in table. 1. The incubation period of the egg ranged from 3 to 7 days with an average of approximately 4 days. The minimum incubation period recorded occurred at temperatures which varied from 85° F. to 90° F., while the maximum occurred at about 75 ° F. Temperatures over the entire period which eggs of this species were observed, September 2 to February 13, varied from an average maximum of 88° F. to an average minimum of 74° F. These were somewhat higher temperatures than those recorded during observation on the incubation period of eggs of *oculata* and *plorabunda*. These higher temperatures probably account for the shorter incubation period noted for *rufilabris*.

The larva completes two molts during the active, or feeding period and undergoes the last molt within a cocoon. The first two larval instars were found to be of about equal duration, each averaging slightly over 2 days. After the second molt the larva feeds for 2 to 7 days and then begins to spin the cocoon in which the prepupal period is spent. From 2 to 6 days after the prepupal period begins the last larval skin is cast and is packed into one end of the cocoon. This skin can be seen through the thin cocoon wall as a dark disk and its presence was used as an indication that pupation had occurred. The duration of the pupal stage was subject to considerable variation but averaged 6.5 days from the 95 pupae observed in the laboratory. The time of development from hatching to adult emergence ranged from 16 to 27 days, and the average for 95 individuals was about 18 days.

All females used in the oviposition studies were confined with males soon after emergence and in most cases

Table 1.—Duration of the developmental stages of three species of *Chrysopa*.

Species and Stage	Number of Records	Duration in Days	
		Range	Average
oculata			
Incubation period	591	3–7	5.2
First instar	92	2–5	3.1
Second instar	92	2–6	2.8
Third instar	88	2–8	3.6
Prepupal	88	4–10	6.2
Pupal	68	8–15	11.0
plorabunda			
Incubation period	203	3–7	4.9
First instar	119	2–5	3.5
Second instar	112	2–5	3.0
Third instar	108	2–6	3.5
Prepupal	108	2–7	3.7
Pupal	103	5–13	8.1
rufilabris			
Incubation period	208	3–7	3.7
First instar	115	2–4	2.4
Second instar	115	2–6	2.3
Third instar	111	2–7	3.0
Prepupal	106	2–6	2.7
Pupal	95	4–11	6.5

Table 2.—Fecundity records on three species of *Chrysopa*.

Species	Number of Records	Preoviposition Period (Days)		Oviposition Period (Days)		Number Eggs Deposited	
		Range	Average	Range	Average	Total	Average
oculata	18	5–32	12.5	5–31	12.1	3322	185.4
plorabunda	20	5–39	13.0	5–61	20.6	639	32.0
rufilabris	17	4–14	8.2	2–30	11.3	531	31.2

mating was observed to occur within the first 2 days of adult activity. The preoviposition period of *rufilabris* was usually somewhat shorter than that of the other two species. The oviposition periods of *rufilabris* and *oculata* were of about the same length but averaged only slightly over half as long as that of *plorabunda*. These data are summarized in table 2.

Seventeen *rufilabris* females deposited 531 eggs in a total of 192 oviposition days. The greatest number of eggs deposited in a 24-hour period by a female of this species was 11. One individual deposited 87 eggs over a period of 27 days.

It may be noted from longevity records presented in table 3 that there was little difference in the length of life of the two sexes.

The newly hatched larva begins feeding shortly after emerging from the egg. There is a cessation of feeding before and after each molt and again just before the cocoon is formed. A summary of feeding records on 16 larvae is presented in table 4.

Chrysopa plorabunda.—As indicated in table 1, the average incubation period of 203 eggs observed in the laboratory was about 5 days. These records were taken from September 11 to February 5, during which time the temperature gradually decreased from 85° F. to about 75° F. In general, the developmental stages of this species were more protracted in duration than those of *rufilabris*. The time of development from hatching to adult emergence ranged from 15 to 29 days, and the average for 103 individuals was 23 days.

Twenty females deposited 639 eggs over a combined oviposition period of 411 days. One individual deposited 85 eggs in a period of 26 days.

This was the only one of the three species in which the females usually lived longer than the males. Three of 14 females under close confinement in the laboratory lived for 110 days or more. One of 14 males lived 102 days while the majority lived only about 35 days.

Table 3.—Longevity of three species of *Chrysopa*.

Species and Sex	Number of Records	Longevity (Days)	
		Range	Average
oculata			
Female	17	6–47	22.9
Male	17	8–47	26.4
plorabunda			
Female	14	31–150	62.5
Male	14	4–102	34.6
rufilabris			
Female	18	8–45	23.6
Male	18	10–43	23.5

Table 4.—Number of cotton aphids consumed by larvae of three species of *Chrysopa*.

Species	Number of Records	Average Feeding Period (Days)	Number Aphids Consumed by Instars Specified			Average Number Aphids Consumed by Each Larva
			First	Second	Third	
oculata	15	11.1	600	1101	2284	265.6
plorabunda	18	11.8	821	1202	1721	208.0
rufilabris	16	8.3	594	974	2734	268.8

Chrysopa oculata.—The duration of the larval stages of this species was similar to that of *plorabunda*. The prepupal period was considerably longer than the active feeding period of the third instar larva, whereas in the other two species, these two periods were of about equal duration. The time of development from hatching to adult emergence ranged from 21 to 38 days, the average for 68 individuals being 27 days.

One of the noticeable differences in the biology of *oculata* and the other two species was manifested in the fecundity of the females. Several of the females under observation in the laboratory deposited as many as 50 eggs within a 24-hour period. Eighteen females deposited 3322 eggs in a total of 218 oviposition days. Several of the females laid over 200 eggs and one individual deposited 921 eggs in a 31-day period.

Eight adult *oculata* consumed 2855 cotton aphids in a period of 10 days. One adult devoured 453 aphids during this time.

Summary.—The biology of three species of *Chrysopa* was studied at College Station, Texas. All three species were found to occur in aphid-infested cotton fields. Two species, *rufilabris* and *plorabunda*, overwinter in this area as adults, while *oculata* probably overwinters as prepupae in the soil. The life history and habits of *oculata* differed from those of the other two species mainly in adult feeding, number of eggs deposited and in the fact that the larvae enter the soil to form cocoons and pupate. Adult *oculata* feed directly on aphids, whereas adult *plorabunda* and *rufilabris* feed only on honeydew or other liquids. The maximum number of eggs deposited by an individual *oculata* was 921, compared with 85 for *plorabunda* and 87 for *rufilabris*. The results of laboratory tests proved the larvae of the three species to be voracious feeders on the cotton aphid.

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SCIENTIFIC NOTES

A Cimbicid Sawfly Feeding on an Ornamental Honeysuckle[1]

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During the first week in June 1955, a request for information on how to control larvae of an insect feeding on an ornamental hedge in Berkeley, California, came to the attention of the author. An investigation in mid-afternoon of June 9, 1955 revealed that the hedge in question was a honeysuckle, *Lonicera nitida* Wilson. The day was cool, overcast and sunless. Despite the fact that no larvae could be found during a half hour search, I decided to return again later that evening since sawfly larvae were suspected as being the culprits, and many species are nocturnal in their feeding habits. After dark, 14 fully grown larvae of a cimbicid sawfly, genus *Zaraea*,[2] were easily found with the aid of a flashlight. They were on top of the hedge canopy, fully extended feeding upon the foliage. When disturbed, the larvae coiled in a tight helix, usually with the anal end uppermost, and when picked up or touched with a blunt object would emit a droplet or so of faint greenish-yellow, slightly viscous liquid. This material was ejected from supra spiracular glands as a gradually enlarging droplet or, on occasions, forcibly in a jet-like squirt which reached an inch or so distance.

Fully mature larvae were over an inch in length when extended, plumbeous-blue above, beautifully marked with yellow, black and white spots and covered with a waxy bloom. A light colored sub-lateral band extended from the head to the posterior part of the body (fig. 1).

The larvae were not gregarious, but were well scattered on both sides of a hedge bordering a 12-foot walkway. Injury could be found, but only after a very careful search and no treatment was warranted or recommended.

FIG. 1.—Mature larva of *Zaraea americana* Cresson feeding upon the ornamental honeysuckle, *Lonicera nitida* Wilson.

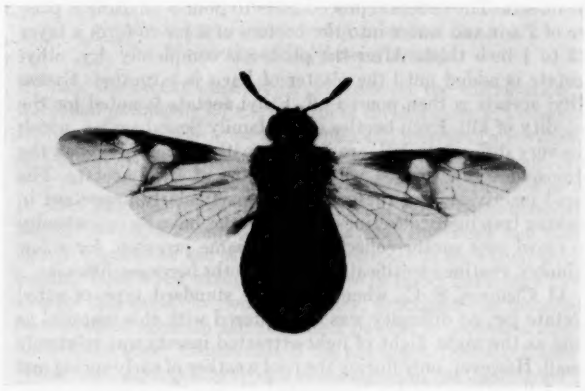

FIG. 2.—Adult female *Zaraea americana* Cresson.

Larvae were brought into the laboratory where they readily fed upon *L. nitida* and *L. japonica* Thunb. Twelve were placed upon a potted *L. nitida* in a nylon mesh sleeve cage to complete their feeding and development. Two were killed in hot water and preserved in alcohol. The living larvae began forming cocoons on June 14, 1955, and by June 22, ten had formed cocoons on the sides of the cage and not on the host plant. Two larvae failed to form cocoons and died. The larvae went through no radical color change except for a slight darkening. When first secreted, the cocoons were a light translucent color, eventually darkening to a dark brown. The cocoons were thin, parchment-like, somewhat oval, but distorted due to being formed in the folds of the cloth sleeve. Bits of debris and pellets of frass adhered to the outside. When later opened the cocoons proved to be formed from two thin layers of brown parchment-like material. These 10 cocoons were then placed in a nylon mesh sleeve cage on the host plant out-of-doors, and permitted to remain there all winter.

Two adult females emerged on March 20, 1956, and proved to be *Zaraea americana* Cresson, a transcontinental species (fig. 2). The remaining eight cocoons were immediately cut open in hopes of securing a pupa, but all eight were adult females and ready to emerge. No males were secured. Adults and larvae are preserved in the Collection of the California Insect Survey, Berkeley.

The only reference I can find on the bionomics of this species is a very brief one by Britton (1925) who reported this species in Connecticut upon a honeysuckle.

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[1] Accepted for publication April 9, 1956.

[2] Hymenoptera, Cimbicidae.

An Improved Ethyl Acetate Jar for Trap Light Collecting[1]

W. F. CHAMBERLAIN[2]

Ethyl acetate has been used for a long time as a killing agent for insects. The common procedure is to pour a mixture of plaster of Paris and water into the bottom of a jar to form a layer 0.5 to 1 inch thick. After the plaster is completely dry, ethyl acetate is added until the plaster of Paris is saturated. Excess ethyl acetate is then poured off. Ethyl acetate is noted for the rapidity of kill. Even beetles of the family Scarabaeidae, which are very difficult to kill, are inactive within a minute when the atmosphere is saturated with the vapors of ethyl acetate. The rapid inactivation of these insects is particularly important in making trap light collections. If the beetles have an opportunity to crawl over moths collected in the same jar, even for a few minutes, routine identification of the moths becomes difficult.

At Clemson, S. C., when using the standard type of ethyl acetate jar, no difficulty was encountered with this material as long as the night flight of light-attracted insects was relatively small. However, only during the cool weather of early spring and late fall can small flights be expected. During the summer a small trap light with a quart jar attachment could be expected to have a layer of 1 to 5 inches of insects by morning. In such cases the standard ethyl acetate jar fails to inactivate the insects rapidly and the moths are unrecognizable within a short time. Not only are the moths' scales lost but often nothing but wing stubs are left of the formerly beautifully colored wings.

In an attempt to prevent the above condition, the usual procedure for the application of the plaster of Paris was slightly modified. After pouring in the mixture of plaster of Paris and water, the jar was turned on the side and rotated so as to deposit the mixture not only on the bottom of the jar but also on the side walls. The best deposit of the plaster of Paris is obtained when the mixture is rather thick but still flowable. The plaster coated jar is then dried in an oven at about 50 degrees C. for 24 hours, and ethyl acetate is applied in the usual manner after a check is made to be sure the plaster on the side walls is saturated. With this type of jar, even large flights of moths and beetles have been successfully collected without damage to the moths. Collections even larger than a quart are possible by treating a relatively tall 2-quart jar.

The apparent reason for the high efficiency of the new type jar is that the fumes of the ethyl acetate are always at a high concentration at the top surface of the layer of insects. With the jars previously used the packing of the insects in the bottom of the jar prevents the fumes from reaching those insects arriving at the trap light later in the night and therefore the specimens are badly damaged.

For best results the jar should be treated with the ethyl acetate each day. The initial treatment required about 10 ml. of ethyl acetate and each day thereafter about 5 ml. If a significant amount of moisture collects in the plaster of Paris, either from the atmosphere or from the insect bodies, the efficiency of the jar is reduced. In such cases the jar can be dried in an oven. Under usual summer conditions this drying is necessary only once every 30 to 60 days. If a third drying is necessary, the jar should be discarded, as usually the collection of oils and dirt reduces the efficiency of the ethyl acetate in inactivating the insects. Jars with the side walls coated with the plaster of Paris have been used for 4 years at Clemson with very good success. A jar of this type is much more efficient than one containing a separate smaller screened jar of ethyl acetate.

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Life History of the Spiny Rat Mite[1] under Artificial Conditions[2]

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Very little is known regarding the biologies of mesostigmatic mites. Life history investigations in this group have been restricted to dermanyssid mites. Early research consisted mainly of observations on the life cycle in cultures simulating the conditions under which the mites live in nature. In the past few years more refined methods have been employed. Bertram *et al.* (1946) described a procedure for rearing the tropical rat mite, *Ornithonyssus bacoti* (Hirst), in tubes except during the protonymphal stage of the life cycle. Skaliy & Hayes (1949) used a modification of this procedure to rear the same species through the entire life cycle within tubes. In both investigations, however, mass rearing methods were employed and the mites were not studied individually. Camin (1953) studied the life history of the snake mite, *Ophionyssus natricis*, individually, utilizing rearing cells attached to snakes.

The present study was undertaken to develop methods for rearing and studying parasitic mites individually, and to contribute to the knowledge of the life histories of mesostigmatic mites, specifically, laelaptid mites.

PROCEDURE.—A stock of *Echinolaelaps echidninus*, obtained through the courtesy of Dr. G. W. Wharton, Department of Zoology, University of Maryland, was cultured in a sheet-metal box having the dimensions of 15×15×15 inches. A 4- to 5-inch layer of wood shavings was placed in the bottom of the box and activated charcoal was mixed with the shavings in the lower portion of the layer. A sheet of ¼-inch mesh hardware cloth was placed over this layer to prevent undue disturbance by the rat host. A white rat, cotton bedding, food, and water were put in the box. A ¼-inch mesh hardware cloth lid covered the top of the box to prevent escape of the rat and allow good ventilation. A strong culture of mites was maintained under these conditions. Mites were easily removed from the culture box with a bulb aspirator.

Mites were reared individually in glass tubes which were 5 centimeters in length and had an outside diameter of 1 centimeter. The ends were closed by placing a piece of silk bolting cloth over each opening and shoving it into the tube with a piece of rubber tubing about 6 mm. long. The tubes were placed in a 150-mm. desiccator over a saturated solution of ammonium sulfate which provided a relative humidity near 81%. The desiccator was placed in an incubator at a temperature of 30° C. Tests had indicated that this would be a satisfactory relative humidity and temperature for the mites.

A life history study was initiated by selecting a female from the culture and placing her in the glass cylinder. As soon as a larva was observed, it was removed to a separate tube. The artificial feeding method described by Cross (1954) was used in this study. The mites were fed on outdated human blood obtained from hospital laboratories. The blood was given cold but this had no apparent effect on feeding. Tubes were changed daily. The mites were observed at regular intervals. Two series of life history studies were conducted, separated by a period of several weeks. Both series were studied under the same conditions outlined above, but there was a difference in the range and average length of the protonymphal stage in the two series. This was the only stage that showed any noticeable variation. We can give no reason for this variation.

BIOLOGY.—This mite is viviparous. The life history consists of four stages: the hexapod larva, the protonymph, deutonymph

[1] *Echinolaelaps echidninus* (Berlese, 1887) (Laelaptidae: Acarina).

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and adult. All stages feed except the larva. No stage becomes distended with food, as do the Dermanyssidae.

The larva usually remains in one place until it molts into the protonymphal stage, but it will move quite rapidly when touched. The larval period is of short duration, ranging from 10.5 to 12.5 hours, with an average of 11.5 hours. Birth of a larva was never observed, but they were found within half an hour after birth.

The protonymph feeds within several hours after ecdysis. In the first study series, involving 33 mites, the protonymphal period ranged from 3.5 to 11 days, with a weighted average of 6.5 days. In the second series, involving 30 mites, the range was 3 to 8 days, with a weighted average of 4.8 days.

The length of the deutonymphal period ranged from 3 to 9 days with a weighted average of 5.3 days. The deutonymph also feeds within a few hours after ecdysis.

Most of the females began to produce young 5 or 6 days after ecdysis; however, in some tubes a larva was not observed until as late as the 17th day following the adult molt. On a number of occasions females were seen to attack their newborn larva and suck all the fluid from its body. The larval skin was carried about by the female for some time. When the skin was dropped and later encountered, the female again seized it with her chelae and carried it about. By this process the larval skin would be wadded into a very small mass which was extremely difficult to detect. It is believed that in those cases where a larva was not observed in the tube of a female for a number of days after the adult molt, the female had killed the larva and its rolled skin was probably left on the silk cloth and was undetected.

An effort was made to get the females to eat their young in the nymphal stages, and also other small arthropods. Animals tried were protonymphs and deutonymphs of the same species (*E. echidninus*), adults and nymphs of other, smaller gamasid mites, eggs and all larval instars of vinegar flies (*Drosophila*), and Collembola nymphs. The females refused to eat any of these. Why they sometimes eat their larvae is not known.

The females will reproduce parthenogenetically, but if given the opportunity will readily mate. Length of life for the adult female ranged from 61 to 91 days, with an average of 78.7 days. Only two longevity records of adult males are available, these are 57 and 76 days.

Limited studies were conducted on the effect of restricted feeding on the longevity of *E. echidninus*. Two types of tests were conducted: in one the mites were allowed to take one meal, in the other the mites were not fed. Unfed protonymphs (15) lived 6 to 7 days. Protonymphs allowed to take one meal (12) lived from 5 to 13 days with an average of 7.3 days. Unfed deutonymphs (10) lived from 7 to 12 days, with an average of 9 days. Deutonymphs taking one meal (8) lived from 14 to 16 days. No molts occurred with only one feeding. Four adults not allowed to feed, lived 7 to 8 days.

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The Availability of Certain Proprietary Adjuvants for Use with the Polyhedrosis Viruses of Insects[1]

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With the recent impetus in research on the distribution of microbial pathogens for the control of certain insects, a need for information on application techniques and materials appropriate to the nature of the agents involved has become apparent. Not only does the particulate nature of the microorganism used seem to offer physical problems differing from those encountered in the distribution of spray emulsions and solutions, but these agents require the use of adjuvants compatible with their living nature. With a few exceptions, studies in insect pathology have usually depended on non-proprietary, proteinaceous materials such as blood albumin and skim-milk powder to provide sticking qualities in water suspensions of the pathogen. Dowden & Girth (1953) used glycogen to reduce evaporation in the application of a polyhedrosis virus of *Neodiprion sertifer* (Geoffr.); Angus (1954) tested methyl-cellulose and found it satisfactory as a spreading agent for suspensions of *Bacillus* spp. used in laboratory tests; and Tanada (1956) used a proprietary agent, Triton B-1956,[2] as an adjuvant for use in field applications of a granulosis virus and *Bacillus thuringiensis* Berl.

This note records the results of laboratory tests of the compatability of several proprietary adjuvants with the polyhedrosis virus of *Colias philodice eurytheme* Bvdl. In addition to methocel,[3] five non-ionic surface-active agents, diesel oil, and combinations of each agent and diesel oil were tested. The agents tested were: Triton B-1956, Triton X-100,[2] Arlacel "C",[4] Span 80,[4] and Tween 80.[4]

To test the effects of the proprietary agents, a water suspension of polyhedra was added to a prepared aqueous 1% solution of the adjuvant and allowed to stand at room temperature. After this exposure, the suspension was applied by atomizer to sprigs of alfalfa which were them fed to test larvae. In addition to treated larvae, two lots of control insects accompanied each test. One lot was maintained with no treatment and the second was fed alfalfa sprigs on which a solution of the agent under test without the virus was sprayed. Each lot, treated and control, was started with 30 larvae, but occasional individuals became lost during the progress of the test. Mortality percentages were calculated on the number of larvae that could be accounted for at the end of the test. All larvae were reared individually.

To test the effect of diesel oil, a water suspension of the polyhedra was added to oil and allowed to stand at room temperature. After this exposure the polyhedra were removed from the oil, resuspended in distilled water, and then applied to alfalfa sprigs. Removal of the polyhedra from the oil was necessary because application of the oil rendered the food unpalatable to the test insects. In addition to the water suspension in oil, a test in which dried polyhedra were added to the oil and allowed to stand at room temperature was run. In addition to the test insects, one lot of untreated controls, and a second lot of controls fed the resuspended material after sterilization by autoclaving were maintained.

The effect of the combinations of adjuvants and diesel oil was tested in the same manner as the effect of the oil. Similar controls were maintained. After initial short-period tests indicated no adverse effects on the virus of any of the agents or combinations tested, suspensions of each were prepared and allowed to stand for several months. Each test was then repeated after this

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[2] Rohm and Haas Co., Philadelphia, Pa.

[3] Dow Chemical Co., Midland, Michigan.

[4] Atlas Powder Co., Wilmington, Delaware.

longer exposure. No adverse effects on the virus as occluded within the polyhedra were found. Exposure, concentration, and mortality data are given in table 1. Inferences as to the resistance or stability *per se* of the virus were not the objectives of the tests, the precise microphysical relationships of the components of the various suspensions are not known. Nor is it known that any of the agents will bring desired results in field application, but all appear to be available for field testing.

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Table 1.—Mortality among larvae of *Colias philodice eurytheme* following feeding polyhedral bodies exposed to various agents.

Material	Exposure (Months)	Test Insects		Control 1[a]		Control 2[b]	
		Per Cent Dead		Per Cent Dead		Per Cent Dead	
		Polyhedrosis	Total	Polyhedrosis	Total	Polyhedrosis	Total
Span 80	8	96.0	100	0	8.7	0	7.7
Triton X-100	10	75.0	96.0	0	20	0	40
Methocel	8	93.0	93.0	0	0	0	0
Arlacel "C"	9	100	100	0	0	0	0
Triton X-100 in diesel oil	10	100	100	0	3	7	10
Tween 80 in diesel oil	8	100	100	4	4	10	10
Span 80 in diesel oil	9	100	100	3	3	0	0
Arlacel "C" in diesel oil	9	89	100	10	10	3	6
Triton B-1956[c]	6	100	100	0	0	0	0
Diesel oil (aqueous suspension polyhedra)	12	86	100	3.3	3.3	6.6	6.6
Diesel oil (dry polyhedra)	14 days	69	100	Not run		3.3	13.3

[a] Control insects fed alfalfa sprigs sprayed with adjuvant without virus or autoclaved test suspension as appropriate for test.
[b] Untreated controls.
[c] Ten insects only in test and each control.

Control of House Fly Larvae in Poultry Droppings[1]

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The evaluation of materials and methods for the control of house fly (*Musca domestica L.*) larvae has received only occasional attention over the years. Early recommendations included the use of fly-tight bins, maggot traps, the compact heaping of manure, or the use of borax (Howard 1926). In recent years several workers (McGovran & Piquett 1945, McDuffie *et al.* 1946, Du Chanois 1947) have reported that BHC and lindane are toxic to house fly larvae. Hadjinicolaou & Hansens (1953) found dieldrin, aldrin, chlordane, and heptachlor effective in laboratory tests and initially so in field trials, but repeated field applications were progressively less effective. McCauley *et al.* (1955) reported on the relative effectiveness of seven chlorinated hydrocarbon insecticides against larvae, pupae, and adults of nonresistant house flies, and Lindquist & Fay (1955) conducted similar studies with eight phosphorus insecticides.

In many areas it is common practice to keep commercial laying flocks of poultry in raised wire-floored pens. When the manure is allowed to accumulate for extended periods under these pens, a serious fly-breeding situation may develop. The manure is usually concentrated in a relatively small area, and within this area fly breeding is restricted to those locations with a source of moisture. Normally the manure dries shortly after the birds drop it, but faulty watering devices, sprinkling systems, or diarrheal birds produce foci of highly productive larval media. Under such conditions an effective larvicide would appear practical.

During the summer of 1955 the authors had an opportunity to investigate several insecticides for the control of house fly larvae in poultry droppings in the vicinity of Fresno, Calif. The test plots contained 24 to 30 square feet of manure under caged white Leghorn hens. The depth of the manure averaged 4 to 6 inches and was fairly constant throughout the various plots. New droppings were, of course, being added continually.

Emulsion sprays of malathion, Chlorthion, Am. Cyanamid 4124, Diazinon, and dieldrin, and water solutions of Bayer L 13/59 were applied at the rate of 10 to 1500 mg./sq.ft. with a variation in volume from 10 to 200 ml./sq.ft. A 3-gallon compressed-air sprayer with a coarse cone spray was used for all treatments.

Results of tests of the 50 ml./sq.ft. applications are presented in table 1. Although these data are selective, they are typical of those obtained with the various insecticides. They indicate Diazinon and Bayer L 13/59 to be superior to the other toxicants as larvicides in regard to initial kill under the conditions of these tests. At 25 mg./sq.ft. Diazinon gave 60% kill and L 13/59 gave 90% in 24 hours and complete kill in 48 hours. At 100 mg./sq.ft. or better both materials caused 100% mortality in 24 hours. With 50 mg./sq.ft. or more L 13/59 killed larvae more rapidly than the other toxicants.

Dieldrin caused complete mortality of first- and second-instar larvae at 500 to 1500 mg./sq.ft., but did not kill all third-instar larvae at even the highest rate. No mortality of larvae was obtained in treatments of 100 mg./sq.ft. or less. Malathion and Am. Cyanamid 4124 gave similar results at 1000 and 1500 mg./sq.ft., but malathion gave higher mortalities at 500 mg./sq.ft. At less than 500 mg./sq.ft. neither material was effective as a larvicide. Chlorthion was the least effective insecticide tested, resulting in only a 70% mortality of larvae at 1500 mg./sq.ft.

Except in the Diazinon plots, reinfestation usually occurred

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Table 1.—Per cent reduction of house fly larvae 48 hours after treatment of poultry droppings at various dosages per square foot.[a]

INSECTICIDE	10 MG.	25 MG.	50 MG.	100 MG.	250 MG.	500 MG.	1000 MG.	1500 MG.
Diazinon	95 (4)	100 (4)	100 (5)	100 (6)	100(7)	100 (9)	100 (9)	100 (9)
Bayer L 13/59	100 (4)[b]	100 (2)	99 (3)	100 (4)	100 (3)	100 (4)	100 (5)	—
Malathion	—	0	0	20[c]	—	95 (4)	97 (5)	93 (6)
Am. Cyanamid 4124	—	—	—	0	—	35	60	95 (4)
Chlorthion	—	—	10	20	—	70	75	70
Dieldrin	—	—	0	0	—	95 (4)	92 (4)	95

[a] All treatments at 50 ml. per square foot. Figures in parentheses indicate days to reinfestation.
[b] Some of the third-instar larvae migrated from plot to alleyway.
[c] 30 per cent at 200 and 300 mg./sq. ft.

on the third or fourth day after treatment with 100 mg./sq.ft. of toxicant or less, or on the fourth or fifth day in plots treated in excess of this amount. Diazinon plots ordinarily required 1 to 2 days longer. However, even with high concentrations of Diazinon, reinfestation occurred not later than 7 to 9 days. Time to reinfestation for a given toxicant at a given dosage was remarkably constant.

The volume of fluid applied within the limits of 40 to 100 ml./sq.ft. did not greatly affect the mortality. In limited trials, applications of 200 ml./sq.ft. appeared to increase the mortality obtained. However, this amount (approximately 2.65 gal./100 sq.ft.) was not considered a practical application with a hand-operated pressure sprayer. Amounts of 25 ml./sq.ft. or less did not result in thorough coverage. Volumes of 40 to 50 ml./sq.ft. appeared to be the most satisfactory for the conditions involved.

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A Counting Plate for Sampling Mite Populations[1]

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The brushing machine developed by Henderson & McBurnie (1943) for estimating mite populations has been put into use by many laboratories in the United States and Canada with satisfactory results. However, in most cases, the counting of the mite populations has involved the use of a complicated grid to subsample the mites caught on the sticky glass plate used with the machine. Morgan *et al.* (1955) discussed these methods and developed an improved counting grid consisting of equal area sections. More reliable estimates of the total population on the plates could be made than with the equal width but unequal area sections.

In mite control studies the comparative population of mites between treatments is the item of interest rather than the absolute population of mites on the leaves. Consequently, any aliquoting procedure should be satisfactory, providing the method is not changed from one plot to another. The problem, then, was to devise a method of aliquoting which provides a valid estimate of the population with the least expenditure of time. Since mite counting is a tedious task, the simpler the procedure the less likely the counts will be in error as a result of operator fatigue or boredom.

By dividing the plate area into equal area sections similar to the design used by Morgan *et al.* (1955) it was determined that more two-spotted spider mites (*Tetranychus telarius* (L.)) were deposited nearer the center of the plates than at the periphery. As indicated by Morgan, the distribution of mites from the center to the periphery of the plate would depend upon the body weight of the mite species and upon other factors. In order to prevent tearing the tender alfalfa leaves under study, it was necessary to use a slow brush speed. This probably reduced the distance to which the mites were thrown by the brushes so that more of them are deposited in the center of the plate than when a higher speed brushing machine is used. It was found, however, that distribution of mites around the plates was relatively uniform. This could be expected since the plate revolves at 40 r.p.m., thereby spreading the mites uniformly on the surface.

The counting procedure that was finally devised used two strips crossing at right angles in the center of the plate (fig. 1). These strips comprise one-tenth of the plate area. With an effective plate diameter of 115 mm. the width of each strip is 4.5 mm. This width just fills the field of a binocular microscope at 18× magnification. After the leaf samples from one plot are brushed and the mites deposited on the sticky plate (a coating of a water soluble solution of polyvinyl alcohol is used to coat the plates), the plate is placed on a counting board on which the dark blue cross strips are laid out on a light colored background. The counting board is pivoted in the center and fastened to a strip of wood which slides in a slot in a wooden base fastened to the microscope stage. The counting board with the plate in place is then slid along the slot centered under the microscope objective. When one cross strip has been counted, the board is rotated 90 degrees (a stop automatically centers it) and the other cross strip is counted by sliding the board in the opposite direction. If a larger aliquot than 10% is desired, the sticky plate can be rotated slightly to a different position and additional counts made.

This method has been used for 4 years (Klostermeyer 1954) and results have been very satisfactory. The labor saved by this method permits taking a larger sample of leaves, more frequent

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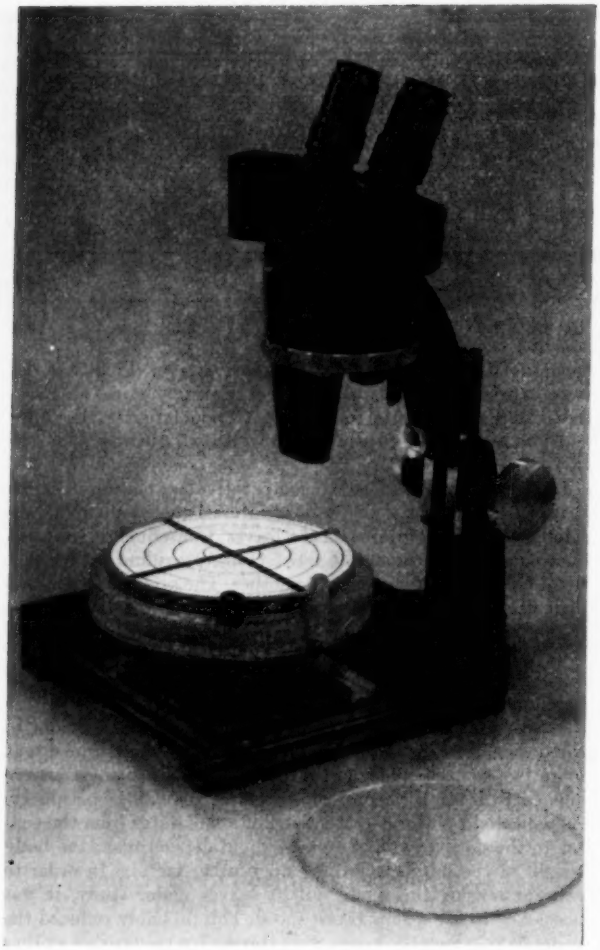

FIG. 1.—Sampling plate for counting mites in conjunction with a brushing machine.

samples, and the use of a larger number of plots than could otherwise be handled.

The brushing machine is also useful for sampling populations of other sedentary, leaf-feeding insects such as aphids, scales, and white flies. There is some error due to incomplete removal of insects from the leaves, but with reasonable care the error is small. Furthermore, unskilled help can be utilized for the brushing process so that the trained personnel can do the counting. Herbaceous crop leaves are more fragile than tree fruits, hence a slower brush speed (600 r.p.m.) has been found desirable for such crops.

When working with two-spotted spider mites on alfalfa it was found that leaf-sampling with the brushing machine was slow and a very large number of leaves was required to obtain a good estimate of the population. Furthermore, predaceous mites were only rarely found on leaf samples, whereas they were abundant in samples taken with a closely woven insect net. Consequently, since 1953, mite samples on alfalfa have been obtained by sweeping. Usually 20 sweeps are taken per plot. The collection of insects and mites is anesthetized by inserting the net end into a large jar containing carbon tetrachloride vapor. The net is then emptied into a special shaker.

A tractor funnel was fitted with a false bottom of 36-mesh saran plastic screen. The lower end of the funnel was lengthened and tapered to fit into a small vial which was fastened on by a spring loaded clamp. A hand crank actuates a shaft having three cams. The cams are linked to the funnel in such a way as to provide reciprocal vertical as well as a horizontal vibratory motion. The shaker is operated for 30 turns of the crank, causing mites to pass through the screen into the vial at the bottom of the funnel. The vial is then removed, stoppered and kept in a portable ice-chest until all samples are collected.

In the laboratory the chilled mites are dumped from the vials over the revolving brushes of the brushing machine. All of the mites are removed from the vial with a camel's hair brush. The mites pass through the brushes and are deposited on the sticky plate. They are then counted in the usual manner. With care in emptying the vials, the distribution of the mites over the plate can be made as uniform as, or more so than, that obtained from leaf samples. Four species of mites occur on alfalfa, and populations of all of them can be determined in this single operation. By using a slightly larger mesh screen, thrips can also be obtained. This method of sampling mite populations could be applied to any similar herbaceous crop that can be swept with a net and to any insect which could be handled in the manner described.

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Control of the Cotton Aphid and Cotton Leafworm with Phosphorus Insecticides[1]

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In 1955 some fields in central Texas became infested with the cotton aphid, *Aphis gossypii* Glov., following heavy spray applications of dieldrin plus DDT or toxaphene plus DDT for late-season control of the boll weevil, *Anthonomus grandis* Boh., and the bollworm, *Heliothis zea* (Boddie). Rains after mid-August following an extremely dry 6-week period resulted in new growth of plants in most fields, creating a favorable environment for the cotton leafworm, *Alabama argillacea* (Hbn.). Heavy cotton leafworm infestations occurred after mid-September, and all succulent fields that had not received treatment for boll weevil control were defoliated by leafworms by the end of the month. Since it was so late in the season, growers made no effort to control them. These conditions afforded an opportunity to test several phosphorus insecticides against the cotton aphid and the cotton leafworm in the vicinity of Waco. Results of one cotton leafworm experiment reported herein were obtained in 1954.

PROCEDURE.—In cotton aphid experiment 1 the plots were eight rows wide and 0.05 acre in size. They were arranged in randomized blocks with three replications of each treatment. Since the plots were small in order that all replicates might be included in the heavily infested portion of the field, one leaf was tagged on each of 25 plants selected at random near the center of each plot. The aphids on these leaves were counted before treatment and 1, 3, 8, and 16 days after treatment. In experiment 2 the plots

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[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Effectiveness of several insecticides against the cotton aphid in field experiments, 1955.

Emulsifiable Concentrate and Pounds per Gallon	Pounds of Active Ingredient per Acre	Average Number of Aphids[a]						
		Before Treatment	After Treatment (Days)					
			1	2	3	8	16	Average
Experiment 1 (Sprays applied at 7.5 gallons per acre on August 9)								
Demeton, 2	0.25	266	0.9	—	0.1	0	0	0.2
Bayer 17147, 2	.25	229	32.6	—	0.6	0.2	0.3	8.4
	.375	229	9.2	—	0.01	0.3	0	2.4
Malathion, 5	.75	336	0.2	—	0	0.01	0.01	0.06
Shell OS-2046, 10.4[b]	.375	259	0	—	0	0.05	0	0.01
Outside check	—	233	268.0	—	212.0	197.0	3.6	170.2
Experiment 2 (Sprays applied at 6 gallons per acre on September 6)								
Bayer 17147, 2	0.25	11.9	0.3	0.1	—	0.2	1.3	0.5
Shell OS-2046, 10.4[b]	.25	22.6	0	0	—	0.1	1.0	0.3
Parathion, 2	.25	21.8	0	0	—	0	0.3	0.1

[a] Per leaf in experiment 1 and per square inch in experiment 2.
[b] Technical material completely miscible in water.

were 2.5 acres in size with no replications of treatments and the counts were made by the customary square-inch method. Treatments are given in table 1.

In the cotton leafworm experiments there were no replications of treatments. In experiment 1, conducted in 1954, the plots were 16 rows wide and the entire rows, 1500 feet in length, in the field were sprayed. In experiments 2 and 3, 0.2-acre plots were sprayed for each treatment. All the leafworm larvae on 25 plants selected at random within each plot were counted just before treatment, 1 and 2 days after treatment, and sometimes after longer intervals. Treatments are given in table 2.

In the large-plot experiments, cotton aphid experiment 2 and cotton leafworm experiment 1, emulsions were applied with an 8-row, tractor-attachment, low-pressure and low-volume spray machine at the rate of 6 gallons per acre. In the other experiments they were applied with a 4-row, tricycle-type, self-propelled machine constructed especially for spraying small areas; an engine mounted on the frame was used to propel the machine and to drive the pump. The total volume of spray applied per acre was 7.5 gallons. In all experiments three nozzles per row were used, and the sprays were applied at a pressure of 60 pounds per square inch.

Table 2.—Effectiveness of several insecticides against the cotton leafworm in field experiments.

Emulsifiable Concentrate and Pounds per Gallon	Pounds of Active Ingredient per Acre	Larvae per Plant Before Treatment	Per Cent Control After			
			1 Day	2 Days[a]	7 Days	13 Days
Experiment 1 (Sprays applied at 6 gallons per acre on August 16, 1954)						
Endrin, 1.6	0.3	13	100	100		
Chlorthion, 4	.375	12	99	100		
Malathion, 5	.25	11	100	100		
Parathion, 2	.25	12	100	100		
Methyl parathion, 2	.25	12	100	100		
Toxaphene, 6	3.0	11	98	100		
Experiment 2 (Sprays applied at 7.5 gallons per acre on September 16, 1955)						
Malathion, 5	0.25	8	100	—	75	—[b]
Parathion, 2	.25	7	100	—	99	79
Bayer 17147, 2	.25	11	100	—	96	31
	.375	11	100	—	96	68
Shell OS-2046, 10.4[c]	.25	11	100	—	36	—[b]
Experiment 3 (Sprays applied at 7.5 gallons per acre on September 22, 1955)						
Bayer 17147, 2	0.25	21	—	100		
Chlorthion, 4	.25	20	—	73		
Bayer L 13/59, 50[d]	.5	17	—	89		
Toxaphene, 6	2.25	20	—	82		
Strobane, 8	2.25	24	—	64		

[a] After 44 hours in experiment 3.
[b] Exceeded initial infestation.
[c] Technical material completely miscible in water.
[d] 50% soluble powder.

Results against Cotton Aphids.—In experiment 1 malathion at 0.75 pound and Shell OS-2046 at 0.375 pound were as effective as demeton at 0.25 pound per acre. Reductions with Bayer 17147 at 0.25 and 0.375 pound per acre were less than with the other aphidicides one day after treatment but approached them on subsequent days. Differences between treatments were not significant.

In experiment 2 there was no appreciable difference in control obtained with 0.25 pound of parathion, Shell OS-2046, and Bayer 17147 for 1 week. The initial infestation, however, was considerably less in the plot receiving Bayer 17147. Reinfestation 2 weeks after treatment was slightly greater following the use of 17147 and OS-2046 than after parathion.

Results against Cotton Leafworm Larvae.—In experiment 1 all the treatments were equally effective 1 and 2 days after treatment.

In experiment 2 complete kill of larvae 1 day after treatment was obtained with 0.25 pound per acre of parathion, malathion, Shell OS-2046, and Bayer 17147, and with 0.375 pound of the last material. Considerable reinfestation occurred after 1 week in the OS-2046-treated plot and some in the malathion-treated plot. After 13 days the infestation exceeded the initial number in both these plots, but fair control continued to be obtained with parathion and with 0.375 pound of 17147 and slight control with 0.25 pound of 17147.

In experiment 3 Bayer 17147 gave 100% kill of a heavy infestation 44 hours after treatment. Control obtained with toxaphene and Bayer L 13/59 was fair, while that obtained with Chlorthion and Strobane was poor. Rain prevented an earlier record, and later records could not be obtained because of migration of larvae from surrounding untreated defoliated areas into treated areas. Although many were killed, migrating larvae were so numerous that all treated areas were defoliated.

Tests with Bait Sprays for the Control of *Drosophila*[1]

HORATIO C. MASON,[2] *Entomology Research Branch, Agric. Res. Serv., U.S.D.A.*

Drosophila infestations are a serious problem for the processors of tomatoes in many parts of the United States. Several species may occur, but *melanogaster* Meig. is generally considered to be the most important. When these flies are present in large numbers in the tomato fields or around the canning plants, there is danger of contamination of the tomato products.

Drosophila flies are readily attracted to certain fruits and vegetables. Because of this characteristic and Steiner's (1952) reported success from the use of bait sprays for the control of fruit flies in Hawaii, tests with baits for the control of these insects were undertaken at Beltsville, Md., in 1954.

PROCEDURE.—Cheesecloth-covered cages 3×3×3½ feet were placed over single unstaked Rutgers tomato plants (fig. 1). Each cage was anchored down and sufficient soil placed around the outside to make it insect-tight at the bottom. When the plants were caged on September 13, they had an average spread of about 3 feet and were approximately 2 feet tall and well set with fruit, many of which were ripe. No tomatoes were harvested from the vines after the test was started. The experimental design was randomized blocks with three replications.

The plants were not treated, but quart strawberry boxes filled with cull tomatoes were sprayed and one box was placed on the ground in each cage within an hour after treatment. A small hand sprayer of the continuous type was used. Dosages were not determined, but the boxes were sprayed until there was a heavy run-off. The three replicates for each treatment were sprayed with the same batch. Two applications were made 15 days apart on September 14 and 29. Different boxes and tomatoes were used for the second application. The ones that received the first application of baits were removed from the cages when those that received the second were added.

Even though the natural infestation in the tomato planting was heavy at the time the experiment was started, the cages were artificially infested with *melanogaster* adults that had been reared in the laboratory. A total of 200 adults was released in each cage on September 14, 22, and 29.

The slit-tomato technique (Michelbacker & Middlekauff 1954) was used for comparing the effectiveness of the treatments. Three tomatoes of nearly the same degree of ripeness and size, each with a fresh vertical slit about 1½ inches long, were placed in each cage at about 4 P.M. on September 16, 20, and 27 and on October 1 and 6. After exposure to the flies for approximately 16½ hours, the tomatoes were removed and the eggs counted in the laboratory under a binocular stereoscopic microscope. A severe windstorm that destroyed most of the cages prevented the taking of additional records.

Heptachlor, malathion, aldrin, and Bayer L 13/59 were tested at 0.1% in a fresh mixture containing 10% of blackstrap molasses, 1% of cider vinegar (acid content 5%), and 0.1% of Fleischmann's Active Dry Yeast Type 1821 in water (all percentages by weight). All the insecticides were wettable powders except Bayer L 13/59, which was a technical-grade powder. The checks were sprayed with water.

RESULTS.—The number of *Drosophila* eggs laid in freshly slit ripe tomatoes sometimes differed greatly between replicates receiving the same treatment. The mean numbers of eggs per replication at different times after each application are shown in table 1.

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[2] The author gratefully acknowledges the assistance rendered by B. K. Dozier, formerly of the Entomology Research Branch.

[3] Rainfall records were supplied by E. E. Wehr, Animal Disease and Parasite Research Branch.

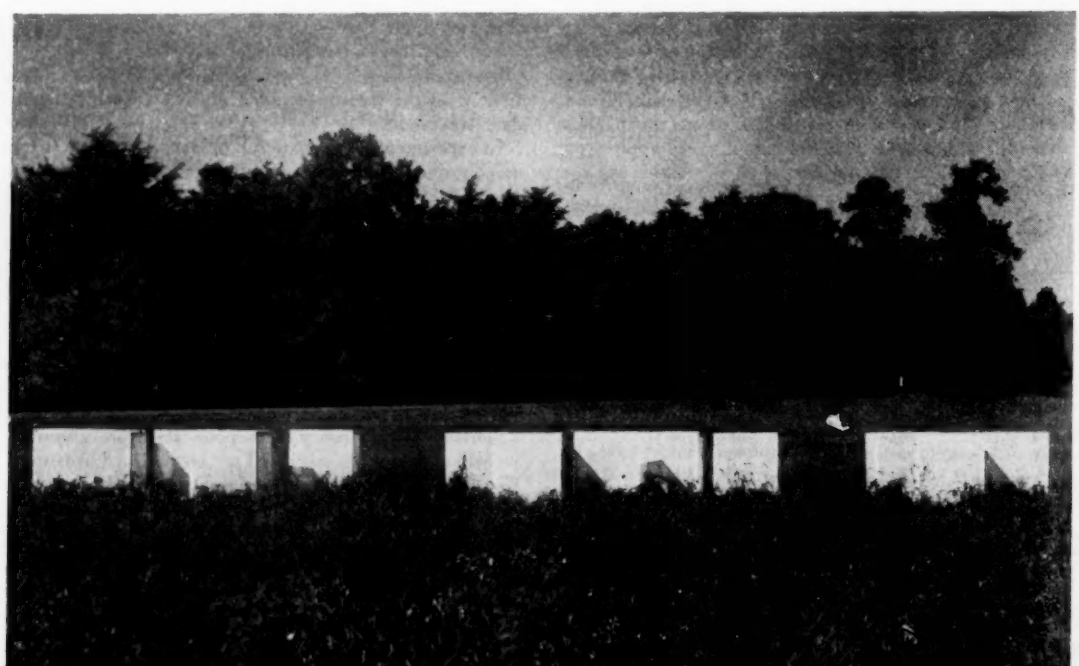

FIG. 1.—Cages over tomato plants as used in bait-spray tests for the control of *Drosophila*.

Table 1.—Mean number of *Drosophila* eggs laid on freshly slit ripe tomatoes in cheesecloth-covered cages over tomato plants in the field.

Insecticide	Days After First Application			Days After Second Application	
	3	7	14	3	8
Heptachlor	33	11	110	59	142
Malathion	227	119	364	240	583
Aldrin	16	29	260	46	55
Bayer L 13/59	6	13	351	144	50
Check	745	365	815	4733	2230
L.S.D. (5%):					
With check	321	180	—[a]	2858	934
Without check	—[a]	—[a]	—[a]	—[a]	376

[a] Not significant.

Three and seven days after the first application all baits showed a significant reduction below the check. Differences between insecticides were not significant, but malathion appeared to be the least effective. After 14 days treatments did not differ statistically.

Three days after the second application all insecticides were giving more than 94% reduction in the number of eggs. Heptachlor, aldrin and Bayer L 13/59 continued to give good protection 8 days after this application. Malathion was less effective but was superior to the check.

Rainfall[3] in general was light during this test. A total of 0.92 inch was recorded near the location of the experiment, the heaviest being 0.38 inch on September 20. The results might have been less promising under conditions of more severe rainfall.

Additional investigations are necessary to determine the value of bait sprays for the control of *Drosophila* under commercial conditions.

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Corn Rootworm Control for Surface-Planted and Listed Corn[1]

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In 1954 over 1¾ million acres of corn land were treated for the control of corn rootworms[2] in Nebraska. Approximately 220,000 acres received insecticide-fertilizer treatment and the remainder was broadcast-treated principally by aerial and ground spray methods. Row treatments were also used to a limited extent.

Between 50 and 60% of the corn crop is listed and the balance is surface-planted. Most of the acreage is broadcast-treated. Thus there are two quite different planting practices and largely one method of insecticide application. At times, however, the broadcast-listing combination as used by farmers in the state resulted in somewhat inferior control as compared with the broadcast-surface planting technique. In 1954 an experiment was set up to determine whether a difference in control of corn rootworm existed between surface-planted and listed corn both of which were broadcast-treated prior to planting.

The experiment consisted of a split plot design in which 8 row plots 50 feet long were sprayed in their entirety with half of each plot (4 rows) being surface-planted and the other half planted by listing. The plots were treated with aldrin, BHC, or heptachlor at the rate of ½ pound per acre. The treatments were replicated four times.

The larvae and pupae in the soil within a circle 12 inches in diameter surrounding each plant were counted. Ten plants in each plot were sampled in this manner; five plants from the listed half and five from the surface-planted half. Lodging and yield were ascertained from records on all the plants in the two center rows of each split plot. Yield was determined by weighing the shelled and dried corn from each treatment by replicates. These data are compiled in table 1.

Table 1.—Corn rootworm counts, lodging and yield of broadcast-treated corn for the two center rows of each split plot.

Treatment[a]	Rootworms Present	Per Cent Lodging	Yield in Pounds
	Surface Planted		
Aldrin	24.0	13.1	18.1
BHC	18.2	2.7	19.8
Heptachlor	15.7	2.0	19.8
Untreated	61.7	21.0	16.2
L.S.D. at 1% level	36.4	6.11	ns
	Listed		
Aldrin	32.7	9.8	21.7
BHC	32.6	1.9	23.6
Heptachlor	23.2	0.8	23.7
Untreated	50.8	11.90	20.7
L.S.D. at 5% level	ns	8.67	ns

[a] Applied at ½ pound per acre.

Insect Counts.—In the surface-planted plots very significant differences in insect numbers occurred between treatments and the check. In the listed plots the results were not significant although the counts in the heptachlor-treated plots were consistantly lower. In general there was a somewhat higher population of insects present in all treatments of the listed plots as compared with the surface-planted plots. This relative increase in the numbers of insects was significantly greater (at the 5% level) only in the BHC-listing combination. Statistical analysis conducted according to Scheffe (1953) indicated no other interactions between insecticide and planting method.

Lodging.—Lodging was determined immediately before harvest by counting the lodged stalks and the total number of stalks in the center two rows of each split plot. Stalks leaning over 30 degrees from the vertical were considered as lodged. Results as indicated in table 1 reveal that significant differences occurred in both surface-planted and listed plots at the 1 and 5% levels, respectively. However, Scheffe's method of analysis indicated no significant differences in lodging between surface planted and listed corn.

Yield.—Yields were determined by harvesting all the ears from the two center rows of each split plot, or a total of 400 feet of row for each treatment. Although the differences were not significant at the 5% level, a general increase in yield was evident in the treated plots. The differences in yield between surface-planted and listed corn were not significant at the 5% level. (Scheffe 1953).

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[2] *Diabrotica longicornis* (Say), *D. virgifera* Lec., *D. undecimpunctata howardi* Barber.

SUMMARY AND CONCLUSIONS.—Except for the BHC-listing combination which resulted in significantly larger insect counts than other insecticide-planting methods, there were no differences between the two planting techniques. It would appear, therefore, that soil insecticides other than BHC should be used for controlling corn rootworm on listed corn. Differences in vapor pressure or other physical or chemical properties of the toxicants may account for the difference in control. The method of soil management which differs between the two types of agronomic practices probably has some effect too. Obviously more work of this nature must be done before final conclusions can be made.

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Effects of Insecticides on Germination and Emergence of Sugar Beets under Greenhouse Conditions[1]

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Insecticides mixed with fertilizer have been shown (Gojmerac & Callenbach 1956) to be an effective way of controlling the sugar beet root maggot, *Tetanops myopaeformis* (von Röder). Observations during 1955 indicated a reduction in stand in rows of beets where insecticides were mixed with fertilizer. The reduction was not serious, the differences being eliminated during the thinning operation.

It is conceivable that under adverse growing conditions, especially during the early part of the season, this "apparent" phytotoxicity could be of serious concern to the grower. To study this problem further, a series of germination and emergence tests was carried out in the greenhouse during the winter of 1955–56. During the course of these studies several interesting observations were recorded as described herein.

MATERIALS AND METHODS.—Germination and emergence tests were performed in greenhouse flats (14″×20″) using Fargo clay soil. The soil was placed in the flats several days prior to planting, moistened and allowed to dry to a workable condition.

Four equally spaced rows were planted in each flat. Fertilizer (0-46-0 at 100 pounds per acre), insecticide, and 30 beet seeds were placed ¼″ deep in each row. One check was placed in each flat. Flats were checked daily; if dry, water was added. The beets were counted at the two-leaf stage.

A cross check was employed to test the possibility of the insecticide spreading in the soil and affecting germination and growth in adjacent rows. Beet seeds and fertilizer were placed in the row as in other tests. The insecticide was placed in a row perpendicular to the rows. If the insecticide was phytotoxic, the effect would be noted on beets at the intersection of the insecticide and beet row. If the insecticide spread in the soil, the phytotoxic effect would be noted on several plants at the intersection of the insecticide-beet row. If there were no observable effects, it was assumed that the insecticide did not spread in the soil, or if it did spread, the effects did not interfere with the germination or emergence.

Dichlone-treated seed from the same lot was used throughout the experiment. Types of insecticides, rates, formulations, and other pertinent data are given in Table 1 together with the results.

Per cent loss in emergence was used as an index to measure phytotoxicity. The sugar beet seed is actually a cluster of seeds. When the seed is prepared for planting it is broken into smaller units. Due to this practice a number of "seeds" may not contain a germ; others may contain several. For this reason, a wide range in germination percentage can be expected—ranges from 75 to 100 may be considered normal.

Table 1.—Effect of chlorinated hydrocarbons and organophosphate insecticides on germination and emergence of sugar beet seedlings.

Insecticide	Formulation	Pounds per Acre	Average Per Cent Loss	Condition of Growth
Heptachlor	25% Vermiculite	1	16	
	25% Vermiculite	5	16	
	40% Celite	1	21	
	40% Celite	5	27	
	25% Attaclay granules	1	20	
Check		0	7	
Heptachlor	25% Attaclay granules	5	24	
	25% WP	1	33	
	25% WP	5	20	
Chlordane	40% WP	1	38	
	25% granules	1	27	
Aldrin	25% WP	1	30	
	20% WP	1	29	
Check		0	23	
Dieldrin	10% Attaclay granules	1	13	
	5% Bentonite granules	1	20	
	50% WP	1	16	
	10% Attaclay granules	3	7	
Check		0	18	
Methoxychlor	25% WP	1	31	
Malathion	25% WP	1	62	
	25% WP	3	53	
	25% WP	3	41	
	15% granules	1	18	
Am. Cyanamid 3911	2% granules	1	38	Stunted
	2% granules	3	79	Stunted
	2% granules	1	57	Stunted
	50% on activated carbon	1	26	Stunted
Dow ET 15	25% WP	3	53	
	25% WP	1	36	
	25% WP	3	57	
Dow ET 14	25% WP	1	37	Curled tops
Hercules AC 528	25% WP	1	12	Stimulated
	25% WP	3	24	Stimulated
	25% WP	3	20	Stimulated
Check		0	25	
		0	37	
		0	8	
		0	23	
		0	25	
Average in chlorinated hydrocarbons			23	
Average in phosphates (except Hercules AC 528)			46	
Average in phosphates			41	
Average in checks			19.8	

Table 1 summarizes the percentage of sugar beets lost in the various treatments. The chlorinated hydrocarbons appeared to be only slightly phytotoxic to sugar beets as judged by the reduction in germination and emergence. The organic phosphates are varied in their effect upon beet seed germination and emergence. Am. Cyanamid 3911 reduced germination and emergence quite drastically. The beets which did emerge were much smaller than comparable checks. Two per cent granulated material appeared to be more phytotoxic than the 50% material on activated carbon. Hercules AC 528 appeared to stimulate germination and growth. The beets emerged a little before the untreated checks and also seemed to grow more vigorously. Beets planted in rows where Dow ET 14 was mixed with the fertilizer emerged; however, the leaves of the plants were distorted and curled. These resembled beets damaged by the herbicide 2,4-D. The other phosphates used in this experiment did not distort the beets, but germination was generally lower than in the checks. Upon termination of the experiment, beets grown in rows con-

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taining phosphate insecticides, except Hercules AC 528, were smaller than comparable checks.

SUMMARY AND CONCLUSIONS.—Under the conditions of the tests conducted, a wide variation in response to plantings of beet seed was obtained. The chlorinated hydrocarbons appeared to be slightly phytotoxic. However, due to the wide variations in germination obtained in the controls, it is believed that these effects were not significant. The phosphates in general were more phytotoxic when employed as soil insecticides. Hercules AC 528, however, appeared to stimulate germination and growth. In this experiment none of the compounds were tested for their insecticidal activity.

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Storage Tests of Water-Dispersible Insecticide Powders at Temperatures from −50 to +55° C.[1]

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A series of storage tests on water-dispersible insecticide powders was begun at Beltsville, Md., in 1954. Powders containing DDT or dieldrin were stored over a wide range of temperatures with variation of humidity. Some of these tests were concurrent with observations begun the previous year on similar materials stored in the field at two locations, one having rather high and the other rather low temperatures.

The storage temperatures at Beltsville were 55°, 10°, −12°, and −50° C., maintained, respectively, in a laboratory oven, a household refrigerator on which a special thermostat had been installed, a cold-storage room, and a subzero freezer chest. Each original sample was divided into subsamples, one for each condition of storage. The subsamples stored at temperatures below freezing were in pint waxed cardboard cartons. Those stored above freezing were in sealed quart fruit jars. There were two jars for each sample at a given temperature. The first jar was simply sealed against change of total moisture from that contained in the powder bed and the atmosphere within the jar. The second jar had a vial attached to the under side of the cap in such a way as to allow air circulation within the jar, and several milliliters of water was put into the vial, with a tuft of cotton to prevent splashing in handling. The object of this arrangement was to provide a practically (if not technically) water-saturated atmosphere. The two moisture conditions are designated as dry and damp. The powders stored under these conditions were one each of a commercial and a laboratory-formulated 75% DDT, a commercial 50% DDT, and a commercial 50% dieldrin.

The locations of field storage were Orlando, Fla., and a mountain pass in New Hampshire, alongside Mt. Washington. The powders under observation were 75% DDT, 50% dieldrin, and 75% lindane, each material being supplied to both stations from the same commercial lot. At both places the powders were kept in drums, in buildings without heat or air conditioning. At the Mt. Washington site the January-February average daily minimum temperature was −8° C. In the Orlando warehouse the July–August average daily maximum was 31°, and the winter temperature never went down quite to freezing.

The methods for checking the condition of the samples at any given time were the average-diameter (air-permeation) test as prescribed in Federal Specification O-I-568 for DDT water-dispersible powder (1951) and Federal Specification O-I-535 for lindane water-dispersible powder (1953), and modifications of the 74-micron wet sieve (maximum-diameter) test and the dispersibility test from the same specifications. The dispersibility test is a 250-micron wet sieve test with a suspension (4% total solids) in standard hard water (342 p.p.m. calculated as calcium carbonate), the suspension being poured through with no agitation or washing on the sieve. The modification in both tests consisted in omitting the heat-and-pressure pretreatment because it would have tended to mask the effects of storage.

In the controlled-condition series the three test measurements were made on each original sample at the beginning of storage, and on each sub-sample at intervals thereafter—a few days at first and then with longer waiting periods as lack of any rapid change was demonstrated. From the field-storage stocks samples were withdrawn twice a year, in fall and spring, and sent to Beltsville for check measurements.

All the DDT formulations in controlled environments were observed for a year, each subsample receiving in this time at least four complete sets of test measurements, for reference against the initial measurements on the original sample. There was no significant physical deterioration under any of the storage conditions except at 55° C. As indicated in table 1, the deterioration at this temperature was slight in dry, but serious in damp storage.

Among the samples placed in controlled storage the dieldrin formulation was unsurpassed in the initial tests. Observations were continued for 3 to 7 weeks, but there was no detectable deterioration, and they were then discontinued, on the assumption from certain earlier experiences that any tendency to aggregation from heat would be marked in the early stages. However, the extent of applicability of this assumption needs further investigation.

The observations on field storage at Mt. Washington covered 2 years, and at Orlando 2 summers with the intervening winter. Each station submitted its samples independently. Since there

[1] This work was conducted in part under funds allotted by the Department of the Army to the Department of Agriculture. Accepted for publication June 13, 1956.

Table 1.—Physical changes in DDT water-dispersible powders after storage for a year at 55° C.

DDT Powder	Under 74 Microns (Per Cent)			Average Diameter (Microns)			Dispersibility (Per Cent)		
		Final			Final			Final	
	Original	Dry	Damp	Original	Dry	Damp	Original	Dry	Damp
75%:									
Laboratory	100	95	22	1.8	2.2	2.6	99	97	65
Commercial	100	100	58	2.4	3.3	3.0	100	79	69
50%, Commercial	100	99	90	2.0	2.1	4.0	86	86	78

was no opportunity for close comparison of the materials before storage began, the original analyses comprised only those required for acceptance of delivery. In no case was there significant evidence of deterioration, except that the lindane from storage at Orlando always ran a little higher in average particle diameter than the specification maximum of 5 microns, and always between two and three times the diameter of the lindane from Mt. Washington. At neither place was there any progressive trend, however.

These preliminary studies indicate that with water-dispersible powders of DDT, dieldrin, and lindane, formulated to standards for ordinary use, serious storage hazards from rigorous climatic conditions do not exist except at high temperatures, and that at high temperatures high humidity is an additional hazard. Points that particularly need further investigation are (1) the possible applicability of these conclusions to the high-suspensibility type of powder, and (2) quick means of detecting potential long-term failure.

Tetranychus marianae McG., A New Pest of Tomatoes[1]

GEORGE P. WENE, *Texas Agricultural Experiment Station, Weslaco*

A mite, *Tetranychus marianae* McG.,[2] was found for the first time in numerous tomato fields in the Lower Rio Grande Valley of Texas during the month of May, 1956. Heavy infestations killed tomato plants in many fields.

Light infestations on tomato plants resulted in little or no leaf discoloration with practically all the mites on the ventral surface of the tomato leaves. As the population became progressively denser the leaves turned a yellowish green in color, and finally a yellowish brown. On the heavier populations mites and their webbings were observed on both the ventral and dorsal surfaces of the tomato leaves, on the stem, and on the fruit. The feeding of the mite caused a silvering on the shoulder of the fruit. This silvery area eventually became russet brown in appearance. Such tomatoes were unmarketable.

On May 22, 1956, these mites were observed migrating from a severely infested tomato field. The tomato plants in the field were yellow due to the mite feeding on the leaves. A number of plants were brown and dead also. On practically every plant there was a webbing covering the terminal 2 inches of branches. Approximately, one-third of the area of these webs was filled with mites, so concentrated that the webs had an orange-colored appearance. A cotton field was located about 30 feet north of this tomato field. Scattered between these two fields were numerous webs on clods of dirt. These webs varied from 2 to 4 inches in diameter and had an orange-colored appearance due to the concentration of mites under the web. Mites could be seen crawling over the ground towards the cotton field. One of these webbed areas was located under a cotton plant where the lower leaves of the plant and others around it were heavily infested with mites. Pritchard & Baker (1955) record the following host plants for this mite: cotton, castor beans, night shade, passion flower and wild lavender.

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[1] Accepted for publication June 27, 1956.

[2] Identified by Edward W. Baker, Agricultural Research Service, Entomology Research Branch, U.S.D.A.

Field Tests with New Materials against Cotton Insects at Tallulah, Louisiana During 1955[1]

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In 1955 several new organic insecticides were tested at Tallulah, La., for control of the boll weevil, *Anthonomus grandis* Boh., the bollworm, *Heliothis zea* (Boddie), the cotton aphid, *Aphis gossypii* Glov., and spider mites.

Three experiments were conducted in plots arranged in randomized blocks, with four replications of each treatment. Each plot was 12 rows wide and of sufficient length to contain about 0.1 acre. All infestation records were made on the eight middle rows. Yield records in experiments 1 and 2 were made on the eight middle rows and in experiment 3 on the four middle rows.

Boll weevil injury was determined by examining 100 squares and bollworm injury by examining 100 bolls in each plot. Counts of aphids and spider mites were made on 100 leaves on 1 square inch per leaf near the center on the underside of the fourth leaf from the top of each of 100 plants. Each infestation record was made at five points in each plot, 20 squares, bolls, or leaves being examined at a point. The points were chosen by the use of random numbers.

In experiments 1 and 3 all materials were emulsifiable concentrates with the exception of Bayer L 13/59, which was a soluble powder. Two formulations of the Bayer L 13/59 soluble powder were compared in experiment 3. Both formulations contained 50% of the active ingredient by weight. Dosages in table 1 are expressed in terms of the active ingredient, and represent the average of 12 applications made at 4- to 5-day intervals. In experiment 1 applications were started on July 13 and ended on September 7, and in experiment 3 they started on July 25 and ended on September 19. Three applications in experiment 1 and one in experiment 3 were washed off by rain within 6 hours. In experiment 2 all materials were dusts. The dosages in table 1 are expressed in terms of the total amount of dust applied, and represent an average of 13 applications starting on July 13 and ending on September 7. Three applications were washed off by rain within 12 hours.

In experiments 1 and 3 the insecticides were applied with a small self-propelled four-row sprayer when the plants were dry, with the exception of two applications in experiment 1, which were made with a man-drawn three-row sprayer. Three number 2 cone-type nozzles per row were used for all applications. In experiment 2 the insecticides were applied with single-nozzle rotary hand dusters early in the morning through August 15 and in the late evening from August 19 on.

The boll weevil was by far the most important insect. In experiments 1 and 2 the weevil infestation developed in early July and remained high throughout the season, but in experiment 3 it did not become serious until early in August. Bollworm moths did not move into cotton in any numbers until after the middle of August. Spider mite populations remained very low throughout the season. Aphids began building up fairly early and were very numerous in some plots by the middle of August.

RESULTS.—The results of these experiments are shown in table 1.

In experiment 1 the plots treated with Bayer 17147 had a significantly lower weevil infestation than any of the others. Chlorthion, Strobane, and Bayer L 13/59 were all significantly lower than the check, but there was no difference between the toxaphene and the check. The Strobane plots had a significantly lower percentage of bollworm-injured bolls than the check, Chlorthion, or Bayer L 13/59. All treated plots had fewer aphids than the check. Chlorthion held the aphid infestation significant-

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[2] The authors were assisted in the field by G. L. Garrison, W. R. C. Craft, E. H. Fisher, C. E. Forrest, Jr., M. A. McDonald, N. P. Mier, H. D. Morton, and C. E. Powell, Jr.

Table 1.—Results obtained with several materials against cotton insects at Tallulah, La., 1955.

Treatment	Dosage Pounds per Acre	Per Cent of Weevil-Punctured Squares		Number per Square Inch		Per Cent of Bollworm-Injured Bolls	Pounds of Seed Cotton per Acre	
		Initial	Average	Aphids	Mites		Total	Gain
			Experiment 1					
		July 12	July 21 to Aug. 23[a]	Aug. 17		Average Aug. 12 and 23		
Sprays (pounds per gallon)								
Untreated		66	69	3.47	0.06	4.5	650	
Chlorthion 4	1.0	63	60	0.10	.01	4.5	685	35
Bayer L 13/59	0.9	70	63	.15	0	4.3	480	−170
Bayer 17147 2	.5	70	54	.29	.01	3.4	760	110
Strobane 6	4.1	73	62	1.45	.02	1.8	1070	420
Toxaphene 6	4.1	70	66	1.54	.02	3.4	1025	375
L.S.D. 5%			5	1.35		1.8	178	
			Experiment 2					
						Average Aug. 12 and 26		
Dusts (figures in per cent)								
Untreated		61	69	2.22	0.38	4.9	860	
Bayer 17147 2.5	17	58	48	0.35	.04	3.4	1805	945
5	17	58	47	.75	.04	3.8	1855	995
DDVP 2.5	16	60	66	.39	.01	4.3	950	90
2.5+Aroclor 10	16	68	64	.67	.01	4.5	910	50
Bayer L 13/59 5	18	60	69	.06	.04	4.6	1235	375
10	19	63	66	.08	.01	3.0	1160	300
Endrin 2	16	66	56	1.13	.21	1.9	1755	895
2+sulfur 40	18	60	55	0.65	.06	3.1	1865	1005
L.S.D. 5%			6.5	1.00	0.18		326	
			Experiment 3					
		July 19	July 28 to Aug. 25[b]	Aug. 15		Aug. 25		
Sprays (pounds per gallon)								
Untreated		22	46	0.30	0	0.8	1400	
DDVP 0.5	0.7	28	43	.06	0	1.3	1300	−100
0.5+Aroclor 2	.7	24	46	.06	0	1.3	1520	120
Bayer L 13/59 Form. I	.7	26	45	.08	0	2.5	1290	−110
Form. II	.7	25	45	.05	0	0.8	1490	90
Dieldrin 1.5	.2	25	43	.36	0	1.3	1450	50
L.S.D. at 5% level				0.18				

[a] Six counts.
[b] Five counts.

ly below toxaphene and Strobane, and Bayer L 13/59 was significantly below toxaphene. These infestation records were not reflected in the yields. The Strobane and toxaphene plots had significantly higher yields than any of the others. The yields in the Bayer 17147 and Chlorthion plots were significantly greater than in Bayer L 13/59 but did not differ from the check.

In experiment 2 Bayer 17147 in 2.5 and 5% dusts gave significantly better boll weevil control than any of the other treatments. Endrin plus sulfur and endrin alone gave significantly better control than DDVP, DDVP plus Aroclor (a chlorinated polyphenyl), or Bayer L 13/59 at either strength. The last four treatments did not differ from the check. There was no significant difference in bollworm-injured bolls between any of the treatments. All insecticides except endrin alone held the spider mite population significantly below the check. DDVP, alone and with Aroclor, and 10% Bayer L 13/59 gave significantly lower spider mite populations than endrin alone. All treated plots had lower aphid infestations than the check. The two Bayer L 13/59 dusts gave lower aphid infestations than endrin alone. The two Bayer 17147 dusts, endrin plus sulfur, and endrin alone gave significantly greater yields of seed cotton than the remaining treatments or check, and 5% Bayer L 13/59 a significantly greater yield than the check.

In experiment 3 none of the treatments gave any control of the boll weevil. There was no difference in the number of bollworm-injured bolls, which was very low in this experiment. Spider mites were not present in this field. DDVP, alone and with Aroclor, and the two Bayer L 13/59 soluble powders all held the aphid population significantly below the dieldrin treatment and the check. There were no significant differences between yields.

In another experiment not covered in these tables, two spray formulations of Am. Cyanamid 3911 were applied three times to the foliage at an average rate of a little over 2 pounds of the technical material per acre. Both formulations gave some control of the boll weevil and prevented an aphid build-up, but did not increase the yield.

Four Pesticides Tested against the Fowl Tick Infesting Turkeys in Feed Lots[1]

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The fowl tick or blue bug, *Argas persicus* (Oken), is occasionally found in turkey flocks in southern California. Tick infestation is often manifested by weakness in the afflicted birds. In

[1] Paper No. 910, University of California Citrus Experiment Station, Riverside, California. Accepted for publication July 3, 1956. The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

addition, direct injuries may be apparent as skin blemishes on thighs, breasts, and sides of the slaughtered birds. These injuries are unsightly and are considered undesirable by the average consumer. As a result, the market price for these birds may be as much as half a cent a pound less than that for unafflicted ones, and this makes a very important difference in profit to the turkey grower.

Bishopp (1919), Stage (1947), and Smith (1952) discuss treatments which can be used against *Argas persicus* but may require repeated applications. The experiment reported here provided the opportunity to test several of the newer organic insecticides against *A. persicus* in southern California and to investigate means of controlling an infestation in turkeys in commercial feeding lots.

MATERIALS AND METHODS.—In September, 1955, a severe incidence of skin blemishes caused by feeding by seed ticks (larvae) of *Argas persicus* was reported on turkeys of a large commercial ranch near Perris, California. Investigation disclosed that the tick infestations were restricted to the wooden feeding troughs used in the pens. These troughs, which were set on the ground, were 8 feet long, 1.5 feet wide, and 2.5 feet deep. Since structures for roosting were not provided, the turkeys crowded up on the troughs to roost. This condition supplied the hiding place by day and the source of food at night required by the fowl tick.

Various devices were tried to trap the ticks and facilitate counting, but since the traps had to be affixed to the troughs, all were rendered useless or were destroyed through the turkeys' pecking at them or clambering over them. Estimates of the number of ticks present were obtained by examining the troughs at night when the turkeys had settled down and the ticks emerged from hiding and moved about. The counts were started about the same time in the evening on each of the counting dates.

Nine range-type pens were used in the experiment. Each pen contained about 800 turkeys and ranged in size from 1.0 to 1.5 acres. A complete pen constituted a treatment unit. One pen was left untreated to serve as a check and 2 separate pens were assigned at random to each of 4 treatments, so that each pesticide treatment was replicated twice.

Four insecticides were selected for testing, each being used in aqueous mixture at a concentration of 1% actual chemical. Sprays of Chlorobenzilate, Diazinon, and Rohm & Haas FW-293 were prepared from the 25% emulsifiable liquid formulations; malathion spray was prepared from the 57% formulation. Power-driven spray equipment provided with agitation in the tank and utilizing a high-pressure reciprocating-type pump operated at 400 p.s.i. was used to mix and apply the spray.

Application of the insecticidal material to the wooden feeding troughs only, was indicated, since these appeared to be the hiding places of the ticks. Before the troughs were sprayed, all turkeys were removed from the pen and the troughs were completely emptied. Special care was taken to remove from the troughs every particle of feed that could absorb any insecticide. All feed spilled around the feeders was raked up and taken away. The spray gun was adjusted to furnish a hard-driving stream at full pressure, and this was directed against the trough at close range to force the material into cracks. A sufficient volume of material was used on each trough to cause runoff. The turkeys were not returned to the pen until the sprayed feeders were completely dry. To minimize absorption of insecticide by the feed, only the amount of feed which would be consumed in a short period was placed in the dry, treated troughs. This precaution was observed for one week following treatment.

DISCUSSION OF RESULTS.—The results of the treatments are given in table 1. The control pen remained infested with ticks throughout the experiment. The experiment was terminated because the time for marketing the turkeys was drawing near, and an interval was necessary for tick-feeding blemishes to heal and disappear. After the last count, all the pens still infested were treated with 1.0% malathion spray according to experimental procedure. Subsequently, the grower reported that at the time of slaughter the turkeys were free of skin blemishes from tick feeding.

Table 1.—Effect of different pesticide sprays when applied at high pressure to wooden feeding troughs in range-type commercial pens for control of the fowl tick on turkeys.

TREATMENT[a]	MEAN NUMBER OF TICKS PER FEEDING TROUGH						
	Before Treatment	After Treatment (Days)					
		1	4	7	11	14	21
None (check)	1.33	20.50	12.20	10.70	6.00	12.70	6.70
Chlorinated hydrocarbon compounds:							
Chlorobenzilate	2.41	42.50	0.83	2.20	0.50	0.91	1.00
Rohm & Haas FW-293	5.00	5.40	0.57	0.43	1.28	2.28	0.71
Organo-phosphorus compounds:							
Diazinon	6.80	0.00	0.00	0.00	0.00	0.00	0.00
Malathion	6.70	0.00	0.00	0.00	0.00	0.00	0.00

[a] Applied at a concentration of 1% actual chemical in aqueous sprays.

The 1.0% malathion and Diazinon sprays gave complete tick control within 24 hours. The 1.0% Chlorobenzilate and Rohm & Haas FW-293 sprays reduced the tick population considerably by the fourth day, but the residual toxicity of these two materials apparently did not persist long enough to clean up the infestation by the 21st day. The results suggest that higher dosages might provide better control by these two compounds.

SUMMARY AND CONCLUSIONS.—Four organic insecticides were applied as aqueous sprays at high pressure to wooden feeding troughs for control of the fowl tick or blue bug, *Argas persicus* (Oken), on turkeys in large range-type commercial feeding lots. Sprays of 1.0% malathion, made from the 57% emulsifiable liquid formulation, and of 1.0% Diazinon, made from the 25% formulation, gave complete control of the fowl tick for the observation period of 21 days following application. Sprays of 1.0% Chlorobenzilate and of 1.0% Rohm & Haas FW-293, made from the 25% emulsifiable liquid, gave good initial kills but failed to clean up the tick infestation. Turkeys from the malathion-treated and Diazinon-treated pens were free from tick-feeding skin blemishes when slaughtered for market.

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The Action of Radiostrontium in the House Fly and the German Cockroach[1]

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Grosch & LaChance (1956) found that radiostrontium caused permanent sterility in the wasp, *Habrobracon*. Similar experiments on the house fly, *Musca domestica*, L., and the German cockroach, *Blattella germanica* L., are herein reported.

The biological half-life of strontium^{89} in these species and the ability of house fly eggs to absorb and retain the element from ingested food has also been determined.

MATERIALS.—Radioactive strontium (Oak Ridge National

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Laboratories) as $Sr^{89}Cl_2$ in HCl was used. Its initial activity was 8.29 mc/ml at the start of the experiments. For the house fly feeding experiment 5 ml. of $Sr^{89}Cl_2$ (41.45 mc) was thoroughly mixed into 5 grams of dried skimmed milk. For the cockroach experiment 3.2 grams of meat-based dog biscuit were ground and impregnated with 10 μl. of above, diluted with water for better dispersion, to give a concentration of 0.083 mc. A low concentration was used in an attempt to overcome the possible repellency to cockroaches of the hydrochloric acid. Water was given separately.

HOUSE FLY TESTS.—Newly emerged house flies, drawn from an insecticide-susceptible strain and reared by standard laboratory procedure, were allowed to feed for 18 hours on the $Sr^{89}Cl_2$-impregnated skimmed milk. After feeding, the flies were separated into two lots. The first, consisting of approximately 100 flies, was allowed to continue feeding on the treated milk; the second lot of 75 was placed on a usual diet of skimmed milk. Ten flies, 5 of each sex, were drawn from the latter lot, anesthetized by carbon dioxide and each placed in a planchet and covered with aluminum foil (4.31 mg/cm²). Their individual radioactivities were determined with a G.M. tube (window 1.4 mg/cm²). They were then individually placed in pint fruit jars and allowed to feed normally on cotton wetted with skimmed milk. The radioactivity of each insect was determined daily for a period of 5 days, when it leveled off.

The counts per minute expressed as percentages of original counts per minute are given in table 1. Correction for radiodecay was not made because of the long half-life of Sr^{89} and the short term of the experiment. From curves plotted from the data in table 1 it was estimated that the biological half-life of the ingested strontium was 14 hours for the male and 16.8 hours for the female flies.

When the flies were 3 days old, each of the aforementioned lots was set up to induce oviposition. Those flies that fed continuously on radiostrontium-milk laid no eggs. The second group, those flies fed for 18 hours, laid many eggs which were radioactive. When counted in the same manner as the flies 19 mg. of eggs had an activity of 105 c/m, an average of 0.42 c/m per egg. The viability of these was 15% as opposed to a usual viability of about 95% for normal flies.

At the end of a two-week period a group of flies was taken from the first lot and allowed to feed on untreated skimmed milk for 3 days. Oviposition was induced and 25 of the eggs thus obtained were counted as before. These were relatively high in radioactivity, each egg having an average activity of 23.5 c/m. Only 2% of the eggs hatched.

COCKROACH TESTS.—Some difficulty was experienced in getting cockroaches to feed on the Sr^{89}-impregnated dog biscuit. It appeared to exert some repellency, possibly because of the high acidity of the $Sr^{89}Cl_2$. Therefore, while 5 adult roaches of each sex were allowed to feed on the Sr^{89}-treated food, only 3 individuals of each sex had consumed enough at the end of 3 days to show discernible radioactivity. The insects were then placed on a normal diet and their individual radioactivities determined periodically in the same manner as the house flies. The results are given in table 2. The biological half-life estimated from curves plotted from these data was 3.25 days after cessation of feeding for the males and 0.95 day for the females.

The experiment was concluded when the percentage of radioactivity leveled off. Since none of the females developed oöthecae during the term of the experiment, it was impossible to determine whether fertility had been inhibited by Sr^{89} feeding.

CONCLUSIONS.—The feeding of radiostrontium to house flies caused varying degrees of sterility. Continuous feeding inhibited oviposition entirely. Feeding for shorter periods allowed for egg laying, but caused a marked decrease in egg viability. This agrees to some extent with the findings of Grosch & LaChance (1956) with *Habrobracon* in which complete sterility was effected, but differs in degree in that 2 to 15% of the fly eggs were viable depending on the length of exposure to the radioisotope.

Apparently fly eggs absorb and retain strontium if it is ingested over a period of time. The absorption rate of the element appears to be relatively slow, radioactivity increasing from 0.42 c/m per egg to 23.5 c/m per egg in 2 weeks.

The biological half-life for those flies that fed for 18 hours agreed closely with that found for phosphorus (0.8 day) by the same method (Babers *et al.* 1956) and also with the findings of Grosch & LaChance (1956) with *Habrobracon* who found the biological half-life to be less than 1 day. There was little difference between the sexes.

Not so, however, with the cockroach, in which there was a definite difference between the male and female animals, 3.5 to 0.95 days. We have no explanation for this difference.

Unfortunately, no conclusion can be drawn as to whether Sr^{89} caused sterility in the cockroach, despite the fact that no oöthecae were found. This may have been due to natural causes.

Table 1.—Per cent of radioactivity at subsequent intervals of house flies fed Sr^{89} for 18 hours.

Days After Feeding	Per Cent Radioactivity	
	Males	Females
1	13.7	26.3
2	9.0	9.1
3	7.3	2.9
5	4.0	1.9

Table 2.—Per cent of radioactivity at subsequent intervals of roaches fed Sr^{89} for 3 days.

Days After Feeding	Per Cent Radioactivity	
	Males	Females
1	77.1	47.5
4	40.7	28.2
5	31.2	26.2
6	28.4	22.5
7	25.5	15.8
8	19.3	12.8

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Ethylene Dibromide Fumigation of Mangoes Infested with the West Indian Fruit Fly at Temperatures Near 55° F.[1]

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Following the reports of the high efficiency of ethylene dibromide fumigation against various fruit flies (Balock 1951, Balock & Lindgren 1951, Richardson 1952, 1955, Shaw & Lopez 1954),

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[2] The test fruit was provided by H. R. Cary, W. R. Sudduth, and H. K. Plank of the San Juan, Puerto Rico, and R. G. Oakley and W. W. Chapman of the New York office U. S. Dept. Agric., Plant Quarantine Branch, and H. E. Warmke of the Agricultural Experiment Station at Mayaguez, Puerto Rico. J. W. Bulger also aided in some of the 1956 gas concentration tests.

this fumigant has been used rather widely in Hawaii, Mexico, Puerto Rico, and the United States. Temperatures of 70° F. or above are required. During the last 2 years West Indian mangoes have been arriving under quarantine at New York City, usually at temperatures near 50° (pulp). They must be warmed up to 70° or above before ethylene dibromide fumigation can be made. Since this sometimes takes considerable time in cool weather, it would be advantageous, in this and probably other quarantine situations, to be able to make effective fumigations near 50° to 60°. Previous tests had indicated that fruit cooled to near 50° (pulp temperature) could be effectively fumigated at air temperatures near 85° (Richardson 1952), but it was still a question whether fumigation was effective when both fruit and air temperatures were near 55°.

Experiments to determine whether ethylene dibromide fumigation is effective at temperatures near 55° were made at Hoboken, N. J., in June and July 1954, and from April through mid-July 1955. Mangoes of the varieties Cambodiana and Totefari infested with West Indian fruit fly, *Anastrepha mombinpraeoptans* Sein, were used. Cuban mangoes of the variety Haydn were also exposed for information on mango tolerance under these conditions. In 1954 no fruit flies emerged in the untreated check mangoes, possibly owing to a previous exposure to cold temperatures before receipt at Hoboken, but the mango tolerance tests gave valuable results. The results of these and the 1955 toxicity and tolerance tests are discussed herein.

PROCEDURES.—Four series of toxicity tests, totaling 23 fumigations, were made in 1955 at 52° to 55° (air and fruit) in 55-gallon tight-drum fumigation chambers (fig. 1) at normal atmospheric pressure. Approximately 990 infested mangoes were used, involving an estimated population of about 4,292 fruit flies. In nine of these fumigations mangoes were also exposed for information on tolerance. Eight other fumigations were made at higher dosages for tolerance data only, and five tolerance fumigations from the 1954 tests were included, a total of 308 Cuban mangoes being used.

Fruit loads varied from 15 to 50% of the fumigation chamber space and usually averaged 20 to 30% with about 30 to 50 mangoes, with an estimated fruit fly population of about 150. The load also included a standard wooden crate (approximately 8 by 18 by 15 inches) containing the test fruit with a supporting wooden stand, a 4-blade 8-inch fan, a vaporizing pan, and a hygrothermograph. The test fruit was always located between the center and top of the drum. Space was left for air circulation, the fan being placed on the floor of the drum to prevent stratification of the heavy ethylene dibromide gas (fig. 1).

FIG. 1—Fumigation drum used in ethylene dibromide tests showing arrangement of crate of mangoes (left center), temperature and humidity recorder (lower center), fan (right center), and electrically heated vaporizing pan (upper right).

The fumigation drums were kept in a small thermostatically controlled room with continuous good fan circulation, at temperatures near 50° to 55°. Two-hour exposures, with fan circulation in the drum for the first 15 to 20 minutes, were used in all tests. The dosages of ethylene dibromide were measured in a pipette and injected directly onto a small electrically heated pan, adjusted so that vaporization was complete within 5 minutes. All fruit to be fumigated was first cooled for 1 to 3 days at 50° to 55°. After fumigation it was placed in cages and kept for 2 to 3 days at 60° and then transferred to an emergence room at 85° for approximately 7 weeks.

Two untreated check batches of fruit were used in each series. One (warm check) received no cool-temperature exposure whatever, but was placed immediately in the emergence room. The other batch (cool check) received the same cool-temperature exposure as the fumigated fruit before transfer to the emergence room. Examinations were made for puparia usually two or three times each week. The average numbers obtained per fruit from untreated mangoes varied from 2.1 to 8.7 for variety Cambodiana and 1.5 to 5.2 for variety Totefari.

EFFECT OF DIFFERENT TEMPERATURE TREATMENTS ON UNFUMIGATED MANGOES.—In three comparisons in two series, the average number of puparia from unfumigated cool-check mangoes differed very little from that from unfumigated warm-check mangoes. In a third series the emergence in the cool-check mangoes was much less, 0.3 as compared with 2.8 in warm-check fruit. In the fourth series no flies emerged in the cool-check fruit, but 5.5 emerged in the warm-check fruit; for this reason results from this series could not be used in the toxicity evaluation.

RESULTS OF TOXICITY TESTS.—In seven tests with dosages of 3 ounces or more and three tests with 2 ounces of ethylene dibromide per 1000 cubic feet, there was complete kill of fruit flies (no puparia). With a 1-ounce dosage there was complete kill in one test and 98% mortality in two. In two tests with ¾ ounce the mortality was 96%, and in two with ½ ounce it was 84 and 78%. The LD-95 was indicated to be near ¾ ounce. In a special test with a 2-ounce dosage, where five layers of excelsior were included as fruit packing, there was slight survival (3 puparia from an estimated 166), probably due to sorption of the ethylene dibromide by the excelsior. This corroborates the findings of Sinclair & Lindgren (1953) at higher temperatures.

The fact that, except in the special test, there was no survival at dosages of 2 ounces or higher indicates that ethylene dibromide is highly effective against this fruit fly at temperatures near 55°. Under larger-scale, more heavily loaded, practical conditions, this dosage will undoubtedly have to be increased.[3] The high efficiency noted at 55° compares fairly well with that noted in tests near 85° with the same fruit fly (Richardson 1952).

Since exposure to cool temperatures alone seemed to cause some mortality, this may have contributed to the high toxicity of ethylene dibromide at such temperatures. Recent work by J. W. Balock in Hawaii (unpublished) indicates that ethylene dibromide is also highly effective against the oriental fruit fly, *Dacus dorsalis* Hendel, in papaya fruit at temperatures down to 60° F., when used at a dosage near 4 ounces per 1000 cubic feet. At 40° and 45° mortalities of 99% or over were also obtained with 4- and 6-ounce dosages.

RESULTS OF TOLERANCE TESTS.—These tests indicated good tolerance of ethylene dibromide fumigation at dosages up to 2

[3] Six practical scale tests made at temperatures near 55° F. during May and June 1956 indicated that a dosage near 4 ounces per 1000 cubic feet would be effective in a 50-cubic-foot tank, using approximately a 25% load of mangoes, packed in regular commercial crates. In three tests in a 2600 cubic foot chamber, with approximately 40 to 45% load of crates of mangoes, the required dosage appeared to be near 12 ounces per 1000 cubic feet. Gas analysis indicated much ethylene dibromide gas was taken up—apparently by sorption in or on the fruit, crates and packing.

pounds per 1000 cubic feet for 2 hours at temperatures near 55°. In two tests at 2 pounds for 4 hours tolerance also seemed satisfactory. Fruit cooled to 50° to 55° and fumigated at 75° with a 1-pound dosage for 2 hours was also satisfactory. No off-flavor or quality reduction was found with any of these dosages. These tolerance results corroborate previous findings on mango fumigation at higher temperatures (Richardson 1952, Shaw & Lopez 1954).

SUMMARY.—Small-scale tests at Hoboken, N.J., in 1954 and 1955 indicated that ethylene dibromide fumigation was highly effective against the West Indian fruit fly, *Anastrepha mombinpraeoptans* Sein, in mangoes at temperatures near 55° F. (air and fruit pulp). A dosage of 2 ounces per 1000 cubic feet for a 2-hour exposure was effective in small fumigation chambers with approximately a 25% fruit load. Mango tolerance also appeared good at this and higher dosages. In practical scale tests made in 1956, effective dosages varied from 4 ounces per 1000 cubic feet in a 50 cubic foot chamber with a 25% fruit load up to near 12 ounces per 1000 cubic feet in a 2600 cubic foot chamber with a 40 to 45% load of mangoes, packed in commercial crates.

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The Asiatic Oak Weevil and Other Insects Causing Damage to Chestnut Foliage in Maryland[1]

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In the Eastern and Southeastern United States there has been a great increase in the planting of blight-resistant chestnut trees resulting in an estimated nut production of several hundred thousand pounds in 1955. Nut production is on the increase as some of these trees are beginning to reach their maximum productivity and younger trees are coming-into-bearing. Much of the nut crop during the past 10 years has been sold as seed and has brought premium prices reaching as high as $1.65 per pound. In 1955 one of the Maryland chestnut orchards produced nearly 8,000 pounds of nuts which sold as seed for 40¢ to 85¢ per pound, the price depending upon the quantity purchased. This same variety of Chinese chestnut brought on the consumer wholesale market, in Baltimore, about 21¢ per pound, but only a small quantity of these nuts have reached this type of market.

In the past 20 to 30 years the bulk of chestnuts for sale in our food stores has been imported from Europe. In 1952, nineteen million pounds were imported. In 1954 there was a decided drop to 14 million pounds, this drop is partially due to two factors; (1) chestnut blight in the European trees and (2) labor difficulties particularly in Italy. Within the very near future most of our American production will go to the consumer markets and the price will have to be in a range where consumer resistance to price will not be the major factor in their sale. This factor is of paramount importance if the chestnut industry in the United States is to survive and grow. The cost of insect control on chestnuts is quite high and necessary if the nuts are to be saleable. Already several orchards in Maryland, Virginia and Pennsylvania are being abandoned because of the expense of chemical control and storage difficulties. There are several insects that must be controlled in order to have good nuts and yields. The control of the two species of chestnut weevils is of greatest importance to the chestnut producers. More work is now being done on their biology and behavior and a re-evaluation made of present control measures. This work will be reported upon at a later date.

The insects to be discussed herein are those that cause foliage damage, namely, the Asiatic oak weevil (*Cyrtepistomus castaneus* (Roelofs)), The Japanese beetle *Popillia japonica*, New., two species of mites and a lepidopterous leaf miner in the family Eriocraniidae.

Cyrtepistomus castaneus (Roelofs) has been a real problem in Maryland chestnut nurseries and orchards. Descriptions of the four stages, life cycle, feeding habits, etc. were reported upon in 1955 by Triplehorn. They were first reported in Maryland in September, 1949 and have increased enormously since that time. The adult weevils are leaf feeders and the larvae are presumably root feeders. Young saplings and branches close to the ground begin to show damage first. This generally occurs in early August. Weevil numbers become increasingly greater as the season progresses and by the middle of September damage is quite obvious even in the top-most branches of the tree. The leaf damage to all species of trees observed has followed the same typical pattern. All the leaf tissue was eaten away leaving only the midvein and its lateral branches. With Chinese and hybrid chestnut trees, growing under forest conditions, it was not uncommon to see as much as 35% defoliation. In some unsprayed orchards damage frequently reached the same level. Very young trees were practically defoliated. Where orchards or young nursery stock are growing near to oak forests or wood lots, damage was always greater to chestnut trees than to the oaks. From several observations of these conditions it is believed that chestnut and chinquapin are the choice host plants. The pest has been observed to feed more frequently on the leaves of species of oak, beech and hickory because of the scarcity of chestnuts. Triplehorn (1955) found 22 species of plants on which these beetles will feed. There is considerable feeding on the green chestnut bur, but the extent of damage has not been ascertained. In several unsprayed orchards it was not uncommon to find anywhere from 6 to 12 of these weevils feeding or hiding between the spines of each bur.

A measure of control of this insect results from the foliage sprays for the large and small chestnut weevil *Curculio proboscideus* (F.) and *Curculio auriger* (Casey), respectively. The Asiatic oak weevil is easy to kill by wettable DDT sprays, and oil fogs containing DDT or lindane. The greatest difficulty occurs in areas where the trees are in close proximity to oak wood lots, for reinfestation occurs rapidly after the residue wears off.

JAPANESE BEETLE.—The Japanese beetle is capable of causing extensive foliage damage. It skeletonizes the chestnut leaf in much the same manner as the grape leaf. Where these beetles are numerous, foliage sprays or dusts are necessary. In Maryland Japanese beetles now rarely cause enough damage to necessitate a spray except in nurseries.

SPIDER MITES.—There are two species of spider mites that frequently cause damage to chestnut foliage on the east coast.

Eotetranychus hicoriae (McGregor) has been found in Maryland and reported to cause sporadic damage. The writer has not found

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this mite, but it was reported by Van Leeuwen in the U. S. Department of Agriculture orchard at Glenn Dale, Maryland. Its host plants are hickory, oak, horse chestnut, pecan and chestnut.

Oligonychus bicolor (Banks) has been the most important species during my observations of the past 2 years. This mite has been found feeding on the upper surface of the leaf and occasionally on the bark of young twigs. Where found they are in large numbers. Eggs are laid along the midrib and lateral veins. They are barrel shaped and rather squatty and adhere very strongly to the upper leaf surface.

On the leaf the mite has a very distinguishable color pattern. The legs and anterior one-third of the body are a light yellowish-orange with a narrow yellowish band along the midposterior dorsum. The rest of the dorsum is dark reddish-brown. Color soon leaves the preserved specimen.

The damage is manifested by leaf discoloration along the veins. They, at first, turn a reddish-brown along the veins and later take on a dingy grayish-rust color, due in part to the molt skins and webs.

O. bicolor is fairly common and its distribution is likely to be found all along the east coast. Garman (1923) in Connecticut wrote that this mite is most common on oak and is also found on chestnut and birch.

Control so far has been easy. Sprays, using nicotine sulfate, parathion, Aramite, or malathion have each done an excellent job of cleaning up mite infestations. One application is generally sufficient.

Mnemonica auricyania (WLSM.).—Is a small moth in the family Eriocraniidae, and frequently causes damage to chestnut foliage in the spring. The extent of damage is not great, at the present, but is increasing under orchard conditions each year.

The moth is similar to a clothes moth in general appearance, and has an iridescent golden bronze color sprinkled with bright metallic purple scales. It lays its eggs during mid-April in the leaves of oak, chestnut, chinquapin and probably others in the oak family. These eggs hatch in about 10 days. The mine often begins as a gallery, but soon expands to a blotch which may or may not involve the part of the leaf where the gallery was at first. The blotch becomes big and bulgy and often covers one-quarter or more of the leaf's total area. The entire parenchyma is eaten out and the mine is equally visible from both sides and so translucent that one may easily see the larvae and the black frass which is deposited in long irregular curled threads. The full grown larvea are 9 to 10 mm. in length, whitish in color and flattened.

The period of mining is very short. It is full grown in about 10 days, after hatching from the egg. It then cuts a small semicircular slit in the upper epidermis of the leaf, drops to the ground and makes a cocoon after it digs a few inches under the surface of the ground.

The moths emerge in early April and flight is weak and irregular. Their attack on foliage is short lived and important only in early spring. No chemicals have been recommended so far for the control of this insect.

OTHER PESTS.—There are several other insects that cause damage to chestnut foliage, such as the filament bearer (*Nematocampa limbata* (Haw.)) and the aphid (*Longistigma caryae* (Harris)), but so far these have been of little or no economic importance in Maryland.

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Chemical Control of Pink Bollworms Overwintering in the Soil[1]

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The pink bollworm (*Pectinophora gossypiella* (Saund.)) overwinters in the larval stage in cottonseed. Infested bolls left in the field constitute the principal hazard for an infestation of the following year's cotton crop. Weather conditions sometimes prevent early destruction of stalks and the plowing under of crop residues. Hence a chemical that would reduce overwinter survival would be helpful in pink bollworm control. Tests were conducted at Brownsville, Texas, in 1944 and in 1953–55, to determine the possibility of reducing the survival of hibernating larvae with soil fumigants and organic insecticides.

Weighed quantities of open cotton bolls infested with pink bollworms were treated and buried in hibernation cages. The cages were equipped with traps, and the effectiveness of the materials in reducing survival was based on emergence records from treated bolls as compared with records from nontreated bolls. Each cage covered an area of 1 square yard. Each treatment was replicated three times.

Table 1.—Pink bollworm emergence from buried bolls treated with a D-D emulsion, 1944.

GALLONS PER ACRE	NUMBER OF MOTHS EMERGING PER CAGE	PER CENT REDUCTION IN EMERGENCE
25.5	53	42.4
51.0	16	82.6
102.0	10	89.1
Check	92	

In the tests with D-D 12 pounds of open cotton bolls were buried in each cage at depths of 2, 4, and 6 inches with 4 pounds at each depth. Two furrows about 1½ inches deep and 19 inches apart were made in the cage, and a 50-50 emulsion of D-D and

Table 2.—Emergence of pink bollworm moths from buried cotton bolls treated with various organic insecticides.

	1953–4			1954–5		
INSECTICIDE	Pounds per Acre	Number of Moths Emerging per Cage	Per Cent Reduction in Emergence	Pounds per Acre	Number of Moths Emerging per Cage	Per Cent Reduction in Emergence
Endrin	8	3.0	96.9	8	7.0	90.7
Parathion	10	4.3	95.6	10	8.3	88.9
Isodrin	—	—	—	10	8.6	88.5
Aldrin	10	6.6	93.2	10	13.6	81.9
Lindane	—	—	—	8	13.6	81.9
BHC (gamma)	6	8.3	90.5	6	23.0	69.4
Heptachlor	10	7.0	92.8	20	26.0	65.4
Diazinon	—	—	—	10	32.0	57.0
Dieldrin	10	13.6	85.1	10	39.6	47.4
EPN	16	10.0	89.7	16	39.0	48.2
Chlordane	—	—	—	21	39.6	47.4
Strobane	—	—	—	20	45.3	39.8
DDT	20	26.0	73.4	20	50.3	33.2
Bayer L 13/59	16	16.0	83.6	—	—	—
	8	23.0	76.4	—	—	—
	4	24.6	74.8	—	—	—
	2	38.0	61.0	—	—	—
Malathion	—	—	—	15	69.0	3.7
Chlorthion	—	—	—	10	112.0	0
Am. Cyanamid 4124	—	—	—	10	79.0	0
Paradichlorobenzene	—	—	—	30	79.0	0
Check	—	97.6	—	—	75.3	—
L.S.D. at 5% level	—	19.6	—	—	21.3	—

[1] Accepted for publication July 20, 1956.

[2] In cooperation with the Texas Agricultural Experiment Station.

water was poured in the bottom of each furrow and immediately covered with soil. With the other chemicals 2½ pounds of bolls were used per cage. An emulsion containing the chemical was sprayed onto the bolls at a rate equivalent to 25 gallons per acre, and they were then buried to a depth of 2 inches.

Pink bollworm survival in the tests conducted with D-D is recorded in table 1, and in those with the other insecticides in table 2. It will be noted that a significant reduction in emergence was obtained from most of the insecticides. Endrin, parathion, aldrin, isodrin, lindane, and BHC were the most effective at the dosages tested.

These tests indicate that the pink bollworm carry-over in the field can be greatly reduced by soil treatment with certain chemicals.

An Emulsifiable Concentrate for Pyrethrum and Similar Insecticides[1]

ROWLAND RICHARDS, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Bailey (1948) and Khan (1951) used a pyrethrum emulsion (New Jersey mosquito larvicide) to make mass collections and population studies of tabanid larvae. They applied the emulsion to the surface of the soil, and as it soaked into the soil, it irritated the larvae and drove them to the surface. Previously it had been necessary to spend a great deal of time and effort digging, breaking, and sifting soil to find horse fly larvae.

The New Jersey larvicide is a coarse emulsion. It was believed that the efficiency of this method of collecting larvae might be improved by the better dispersion of pyrethrum in a fine emulsion.

Many emulsifiable concentrates had been made at the Kerrville, Texas, laboratory, using several emulsifiers recommended by the manufacturers for emulsifying pyrethrum. However, none produced emulsions that both dispersed spontaneously and retained stability for any appreciable time. Fine emulsions of kerosene solutions of toxaphene were known and specifications for emulsifiable concentrates were given by the Entomology Research Branch (1954). A blend of nonionic and anionic surfactants, CTX-54,[2] produced such an emulsion. However, that emulsifier was not completely soluble in kerosene alone, indicating that the presence of the chlorinated hydrocarbon insecticide contributed in part to the solubility of the emulsifier in kerosene. Several chlorinated hydrocarbons were added to the pyrethrum-kerosene system, and four Aroclors[3] (chlorinated polyphenyls) were the most satisfactory. Complete miscibility of the formulations was effected by adding the Aroclor to the pyrethrum system. The following concentrates were made, each incorporating one of the four Aroclors: pyrethrins 1% (5 parts of a 20% pyrethrum extract), emulsifier 11% (CTX-54), Aroclor 55%, and kerosene 29%. In cool hard or distilled water all gave complete spontaneous dispersion. The resulting emulsions remained well dispersed for a 14-day observation period. After several days' standing, only slight bottom creaming was noted, and this creamed material was readily resuspended in 10 inversions of the test container, a 100-ml. graduate. The excellent emulsifiable characteristics were not affected by the addition of 10% of piperonyl butoxide.

Two special formulations were made for use in studies of tabanid larvae in the field: (a) pyrethrins 1%, piperonyl butoxide 10%, CTX-54 11%, Aroclor 1248 33%, in deodorized kerosene (all percentages by weight); and (b) the same formulation without piperonyl butoxide.

Anthony (1956) has used these concentrates in field studies of horse fly and deer fly larval populations in Florida and found them helpful under certain conditions.

In another series of formulations the pyrethrins content was increased to 5%, the amount of kerosene was decreased, and the proportion of emulsifier and the Aroclor was maintained at the same level. Excellent emulsifiable characteristics resulted.

To determine how much Aroclor was needed to produce good emulsifiable characteristics, five additional formulations were made. The pyrethrum (20% extract) and the emulsifier were held constant, being 25 and 10% by weight, respectively, and the Aroclor content 50, 40, 30, 25, and 10, in kerosene to make 100%.

Only the first three formulations were considered satisfactory. Excellent spontaneous dispersion was effected and was maintained for a 14-day observation period, when the room temperature ranged from 35° to 80° F. On the basis of this evaluation, at least 30% of Aroclor must be incorporated into this system to insure good emulsifiable characteristics.

When a formulation containing 10% of pyrethrins, 40% of Aroclor 1221, 10% of CTX-54, and 40% of kerosene was diluted to form an emulsion containing 0.05% of pyrethrins, the dispersion was spontaneous and the stability good.

In preliminary field tests, several formulations of pyrethrins, allethrin, or cyclethrin were applied to test animals in 0.05% emulsions. The presence of an Aroclor in the formulation did not have any visible adverse effects on dairy- and beef-type cattle, and there did not appear to be any extending or masking of the insecticidal properties of the materials.

The inclusion of an Aroclor in these emulsifiable concentrates has already been useful in tests at Kerrville with these three insecticides in combination with various candidate synergists, antioxidants, and/or extenders, and it has been possible to compare some of the candidate materials not otherwise soluble in the insecticide-kerosene-emulsifier system. Comparisons have been made without having to change any of the proportions of the basic components in the formulations. No special formulation problems have resulted.

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[1] Accepted for publication July 3, 1956.

[2] Produced by Wm. Cooper & Nephews, Chicago, Ill.

[3] Aroclor 1221, 1232, 1242, 1248, products of Monsanto Chemical Company, St. Louis, Mo.

EDITORIALS

Precision in Scientific Writing

Upon scientists and technologists alike, the vast development of modern science has imposed heavy demands and grave responsibilities. Explorations now under way in the natural, social, and applied sciences must be recorded and transmitted. But the scientist is no longer at liberty to direct his attention exclusively to his fellow-specialists; he must also reach administrators, statesmen, policy-makers, boards, and other scientists in other fields. Increasingly he must interpret for the general public the discoveries that emerge from the laboratory to transform our daily lives. Even in the most practical affairs the written word grows in volume and significance. Efficient operation of commerce, agriculture, industry, and government requires skillful scientific and technical reporting. No longer can the report be considered as mere adherence to a rigidly prescribed pattern; it must be viewed as the product of profound investigation and analysis, of discernment and discrimination.

Of course traditionally and historically, scientific writing is considered as the literature of science as distinct from belles-lettres. But the scientist frequently must direct his writing to scientists outside his specialty, as well as to the general public, so scientific writing is a broader term than technical writing, which includes only the writing the scientist does in addressing fellow specialists or in connection with technological applications of scientific principles. Factual writing in the areas outside the natural sciences in a broad sense likewise may be termed scientific when it submits to the disciplines of method, objectivity, accuracy, clarity and precision. So, on the whole, it may be truthfully said that scientific writing in the highest sense taxes the resources of language.

It has been long realized by the Editorial unit of this *Journal* that the whole subject of scientific writing presents some very real and some very large problems to many contributors, and that all too many of them experience "agonies of composition" in attempting to communicate their findings. Since, of necessity, much of this suffering also must be shared Editorially, it is with particular satisfaction and pleasure that the attention of all such individuals can be directed to a recently issued, extremely helpful volume entitled "*Scientific Writing.*" Written by M. R. Emberger and M. R. Hall, of the University of Louisville, it is under the general editorship of W. E. Britton, of the University of Michigan. (8vo., cloth, 468 pp., illus., N. Y., Harcourt Brace & Co., 1955, $4.50.) The subject has been divided therein logically into three general sections. In the first of these, (Chapters 1 through 6) there appears great emphasis upon the intellectual activity that must precede composition, and it deals with such matters as Scientific Method, the Problem Concept, Definition and Terminology, Collecting Data, Methods and Applications of Analysis, and Interpretation. In the second section, (Chapters 7 through 9) much attention is given to the various problems of communication. It is emphasized that writing for the expert requires care and precision, but that writing for the non-specialist presents vocabulary obstacles that at times can be surmounted only by considerable effort and ingenuity. The subdivisions include discussion of Scientific Style, Directing the Paper to the Reader, and the Techniques of Exposition. The third section, (Chapters 10 through 15) treat of the elements and structure of various types of papers, including the report, the review, the thesis, the research paper, the abstract, the case history, and the book review. Considerable attention also is given to the format of the scientific paper, uses, practices and variations in documentation, and in types of illustrations in all the more common types of papers. Other features of value are the bibliographical footnotes and the study suggestions provided for each chapter and the wealth of illustrative material throughout the text and appendices.

When it is remembered that the average scientific writer frequently is attempting to enlarge or extend the boundaries of his subject by excursions into unknown or into debatable ground, it would be difficult to attempt to estimate the great and far-reaching usefulness to him of a work of this kind.

J. S. Wade

Towards Accuracy and Precision

The Journal of Economic Entomology was established to serve as a medium in which the members of the American Association of Economic Entomologists might publish the results of their research. For such reports to attain the greatest usefulness, they must be readable but above everything else they must be accurate and precise. Precision writing not only means accurate reporting of the facts that have been developed but each word and phrase must have precise meaning.

It is fundamental that the insect which is the subject of experiment must be correctly identified. Everyone agrees with that dictum, but there seems to be a tendency to use short cuts that may reduce the precision of the account. Failure to be exact is the chief concern of this article.

The scientific name is the typical Linnean binomial. However, that is not complete without the author's name. The complete scientific name need not be used throughout the article and often a common name may be substituted. In this Journal the practice quite generally follows the rules but the same individuals who report their research in this periodical frequently must write, for popular consumption, summaries of their findings in leaflets, circulars and extension type bulletins. In this type of writing the need for precise writing is just as important. However, the tendency seems to lean towards elimina-

tion altogether of the scientific name in about 60% of the popular writings. In another large percentage authors use their own coined common names, disregarding the approved common names. However, in writing for any Society publication, it should be remembered that in using common names it is mandatory to use only the names adopted by the Entomological Society of America. The American Association of Economic Entomologists recognized the need for the adoption of popular or common names 10 years before the Journal of Economic Entomology was first published. Some of the entomologists who were prominent in developing and having adopted lists of common names were F. M. Webster, Herbert Osborn, Edith M. Patch, Arthur Gibson, J. A. Hyslop, C. F. W. Muesebeck and many others of equal prominence. The number of names and the correct methods for developing common names has taken many years and the joint consideration of the entire membership of the two major societies. The latest list was published in the Bulletin of the Entomological Society of America, Volume I, Number 4, December 1955. Other lists have been published by the Association of Applied Biologists in England, the Quebec Society for the Protection of Plants, and the Commonwealth Scientific and Industrial Research Organization of Australia.

After all this travail it would seem only common sense to use the results of so much careful thought; but it is not sufficient to use the adopted common names unless each name is connected clearly with a specific scientific name. Some entomologists are of the opinion that the inclusion of a scientific name in a popular account will be resented by the farmer or other laymen. If this is correct, which we doubt, the difficulty can be overcome by placing the complete scientific name in a footnote thus definitely accounting for the identity of the insect under discussion. Thus even though a coined common or colloquial name may be used, disregarding the published lists of approved common names, the use of the complete scientific name in the text, or in a footnote rescues the account from the realm of inexact writing and gives it precision.

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